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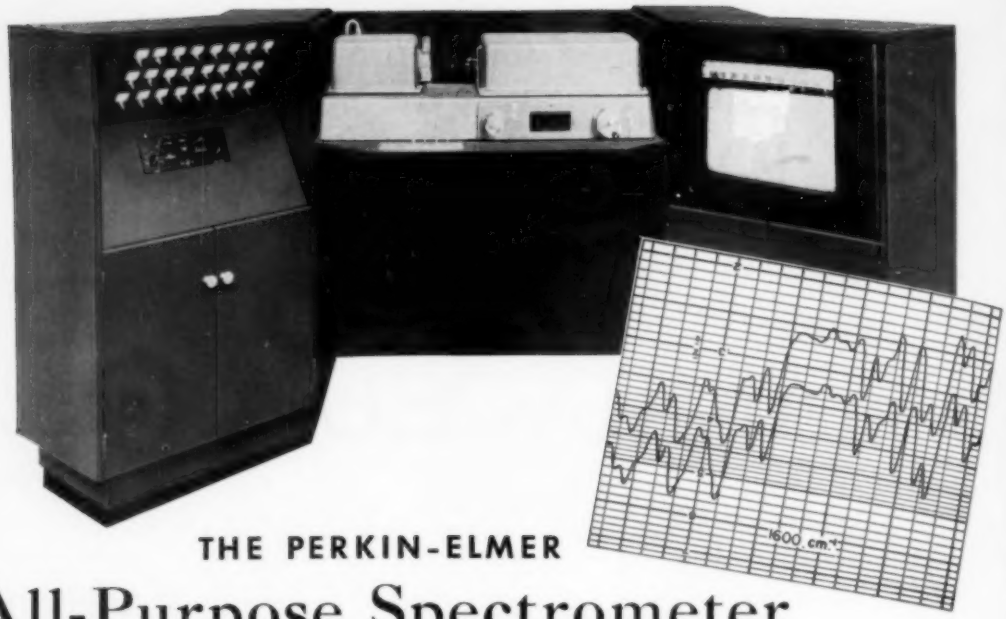

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Streptomycin—Origin, Nature, and Properties*,†

By SELMAN A. WAKSMAN and ALBERT SCHATZ

INTRODUCTION

THE story of streptomycin is the story of a search for an antibiotic substance capable of exerting a bacteriostatic and bactericidal effect upon Gram-negative bacteria, of a substance active against these organisms not only in the test tube but also in the animal body, yet not very toxic nor exerting otherwise undesirable effects upon the body, a substance that would not be inactivated by the body fluids, and that would, therefore, offer chemotherapeutic potentialities. The Department of Microbiology of the New Jersey Agricultural Experiment Station, Rutgers University, first began a study in 1939 of the production of antibiotic substances by microorganisms. These studies resulted in the isolation of a number of antibiotic substances, namely: (*a*) actinomycin, a highly toxic compound; (*b*) clavacin, more active against Gram-negative bacteria but also toxic; (*c*) fumigacin, not so toxic, but not very active; (*d*) chaetomin, nontoxic, but inactive *in vivo;* (*e*) micromonosporin, with little promise as an active agent; (*f*) streptothricin, a substance combining certain desirable properties, but still somewhat toxic, and finally, (*g*) streptomycin, which forms the subject of this paper.

In addition to the above, other antibiotic substances studied by different investigators were also isolated and tested. These included pyocyanase, penicillin-like compounds, and products of spore-forming bacteria. Some thousands of actinomycetes, hundreds of fungi, and many bacteria were isolated from normal soils and from enriched soils, from composts, manures, and peat bogs, as well as from other natural materials. Special methods were devised for the isolation of antagonistic organisms, and techniques were developed for the production, isolation, and study of the active antibacterial substances, the antibiotics. The isolation of streptomycin must, therefore,

* Received Sept. 6, 1945, from the New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, N. J.

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be looked upon as the result of extensive surveys, detailed analyses and numerous tests, in which many collaborators have participated, and to whom a good part of the credit is due.

ISOLATION OF STREPTOMYCES GRISEUS

In the search for antagonistic microorganisms, especially organisms that are active against Gram-negative bacteria, the actinomycetes were found (1, 3, 4) to offer extensive potentialities, 20 to 30 per cent of all the organisms tested possessing marked antibacterial properties. These cultures were either isolated from different soils and composts or were taken from the type collections and tested for their ability to inhibit the growth of bacteria. Only a very few of these cultures offered potentialities as producers of antibiotic substances that could be used for chemotherapeutic purposes. The first among these was streptothricin (2, 11), which was active not only *in vitro*, but also *in vivo* (5, 6), and appeared to offer promise in the treatment of diseases caused by Gram-negative bacteria. Upon subsequent investigation, however, streptothricin revealed certain serious pharmacological disadvantages. Purified preparations exerted a delayed toxic effect upon animals (9, 13, 42); in addition, certain Gram-positive as well as Gram-negative bacteria were found to be naturally resistant to fairly high concentrations of this antibiotic (41).

The limitations of streptothricin, combined with the information thus gained in the isolation of potential chemotherapeutic agents, suggested the desirability of searching for antibiotic substances which might be free of the undesirable properties of steptothricin and which could safely be used for treating diseases caused by Gram-negative bacteria. Simultaneously, studies were initiated of the formation by microorganisms of antibiotics active against pathogenic acid-fast organisms. The marked resistance of mycobacterial diseases to chemotherapy, and the ineffectiveness of penicillin and other antibiotics in the treatment of such diseases were well known. The fact that certain fungi, notably *Aspergillus fumigatus*, and bacteria were found (49) capable of suppressing the growth of *Mycobacterium tuberculosis* suggested the advisability of searching among various groups of microorganisms for agents that could be utilized for chemotherapeutic purposes.

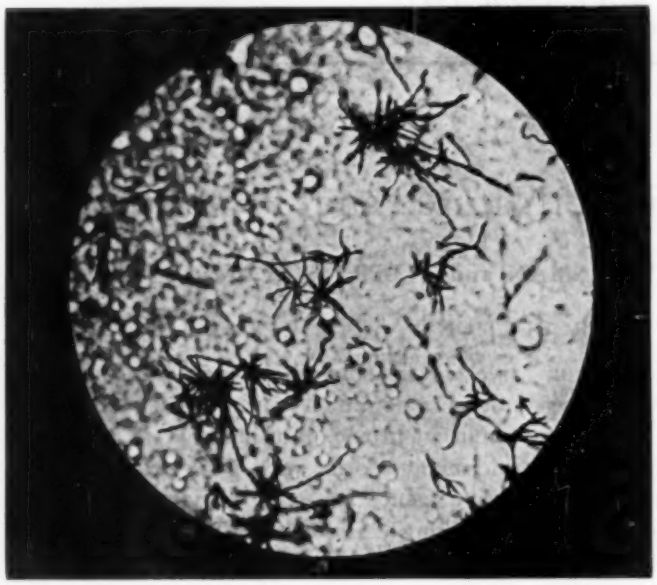

Fig. 1.—*Streptomyces griseus* x 200. Streptomycin - producing strain, showing spore-forming aerial mycelium.

These studies resulted in the isolation of an actinomyces (17, 19) which was found capable of producing, in certain media, an antibiotic substance that apparently possessed many of the desirable antibacterial and pharmacological properties. This organism was similar, in its appearance, cultural characteristics, and morphology, to *Actinomyces griseus* isolated from the soil some twenty-eight years previously in this laboratory (Fig. 1). The antibiotic substance produced by the freshly isolated strain of this organism, in a medium containing meat extract, was very similar to streptothricin; it differed from the latter, however, in its greater activity against certain Gram-negative bacteria, notably *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Serratia marcescens*, as well as against those aerobic spore-

forming bacteria, such as *Bacillus mycoides*, which were resistant to streptothricin. This substance was designated as streptomycin, the name being derived from the generic designation given to the sporulating and aerial-mycelium producing group of actinomycetes, namely *Streptomyces*.

as brought out in Table I. Corn-steep liquor can take the place of meat extract, although the problem of purification of the streptomycin is thereby complicated. The nitrogen and carbon sources are more or less nonspecific, starch and glycerol being satisfactory substitutes for glucose, whereas un-

TABLE I.—INFLUENCES OF NITROGEN SOURCE AND MEAT EXTRACT ON THE PRODUCTION OF STREPTOMYCIN IN STATIONARY CULTURES[a] (19)

Nitrogen Source	Meat Extract, Gm./L.	Days of Incubation: 7 γ	7 pH	9 γ	9 pH	14 γ	14 pH
Sodium nitrate[b]	0	0	5.2	0	...	0	5.4
Sodium nitrate	0.5	2	6.7	3	6.8	1	6.9
Sodium nitrate	5.0	24	7.4	65	7.7	52	7.9
Peptone[c]	0	4	7.9	4	8.1	2	8.0
Peptone	0.5	14	7.6	25	7.8	14	7.8
Peptone	5.0	37	7.5	75	8.0	60	8.3
Glycine[c]	0	0	6.0	0	...	0	5.9
Glycine	0.5	58	7.7	26	7.8	17	8.3
Glycine	5.0	75	8.0	85	8.4	28	8.6

[a] Activity expressed in terms of micrograms of pure streptomycin per 1 ml. of medium.
[b] 2 Gm. per L.
[c] 5 Gm. per L.

TABLE II.—GROWTH OF *S. griseus* AND PRODUCTION OF STREPTOMYCIN (17)

Shaken Cultures: Incubation, Days	γ	pH of Medium	Growth,[a] Mg.	Stationary Cultures: Incubation, Days	γ	pH of Medium	Growth,[a] Mg.
2	10	7.8	270	3	6	7.7	73
3	70	7.7	185	5	12	7.8	171
4	60	7.8	...	7	53	7.9	163
7	70	8.2	...	9	..	8.3	264
				12	55	...	...

[a] Weight of dry mycelium produced by *S. griseus*.

Two cultures of the streptomycin-producing strain of *Streptomyces griseus* were obtained, one (No. 18-16) from a heavily manured field soil and the other (No. D-1), a somewhat less active form, from the throat of a chicken. The almost simultaneous isolation of the two cultures and the appreciable difference in activity at the time of isolation are evidence that they were independent isolations, rather than that one was obtained as a spore contaminant of the other.

PRODUCTION AND ACTIVITY OF STREPTOMYCIN

The composition of the medium was found to have a marked effect upon the production of streptomycin, in contrast to the formation of streptothricin in fairly simple media. Streptomycin production depends largely upon the presence in the medium of a certain organic substance supplied by meat extract, hydrolyzed and hydrolyzed casein, various tryptones, amino acids, and sodium nitrate are approximately as effective as peptone. A medium was finally adopted, consisting of

Glucose	10 Gm.
Peptone	5 Gm.
Meat extract	5 Gm.
NaCl	5 Gm.
Tap water	1000 ml.
Final pH	6.5–7.0

The rate of growth of the organism and the course of production of the streptomycin in shaken and in stationary cultures are brought out in Table II. Two methods were used for measuring the antibiotic activity of the culture, namely, the dilution method, as determined by the agar streak, and the diffusion or cup assay method. In shaken cultures, maximum growth occurred in two days, accompanied in two to three days by maximum activity. In stationary cultures, growth was more gradual, the maximum

being reached in nine days. The medium became alkaline in both cases.

The selective effects upon different bacteria, or the bacteriostatic spectra of streptomycin and streptothricin, are presented in Table III. The spectra emphasize the differences in the antibacterial properties of the two antibiotics. With crude preparations, and with *E. coli* as the test organism, streptomycin is shown to have virtually the same activity as streptothricin against *Bacillus subtilis*, *Staphylococcus aureus*, and certain strains of *Salmonella* and *Pr. vulgaris;* it is somewhat less active against *Shigella*, and much more active against *B. mycoides*, *Mycobacterium phlei*, *M. tuberculosis*, *S. marcescens*, *Ps. aeruginosa*, and others.

TABLE III.—BACTERIOSTATIC SPECTRA OF STREPTOMYCIN AND STREPTOTHRICIN[a] (19)

Test Organism	Streptomycin	Streptothricin
Bacillus subtilis	25,000	30,000
Bacillus mycoides	7,500	<100
Staphylococcus aureus	1,500	2,000
Mycobacterium phlei	10,000	5,000
Mycobacterium tuberculosis (Human, H37)	3,000	100
Escherichia coli	2,000	3,000
Shigella gallinarum	2,000	5,000
Salmonella pullorum	3,000	5,000
Serratia marcescens	2,500	200
Pseudomonas aeruginosa	300	50

[a] Comparative activity of two crude preparations in dilution units, as determined by plate method.

The strains of *Streptomyces griseus* that produce streptomycin are not absolutely stable, but undergo marked variation (39). On plating out the active culture of this organism, certain variants can be obtained which differ considerably in their ability to produce streptomycin. They produce no aerial mycelium, form no streptomycin, undergo rapid lysis, give rise to an acid reaction, and yield a viscous broth. The inactive variants are sensitive to the antibiotic action of streptomycin, whereas the active mother culture is highly resistant to streptomycin. However, both the active culture and the inactive variants are alike in many of their cultural characteristics, such as lack of dark pigmentation on organic media, proteolytic action, and hemolytic capacity. By proper culture and selection, the inactive asporogenous strain of the organism can be made to revert to an active sporulating form which will then produce the typical streptomycin. The inactive variants, not forming any aerial mycelium, are thus markedly different, in certain physiological properties, from the mother culture, although the general cultural properties are similar. The antibiotic potency of the active cultures of *S. griseus* is fairly stable. With the same medium and under the same cultural conditions, the comparative differences among different strains remain approximately the same. A highly active strain retains its relatively superior potency, whereas a poor strain usually remains a weak producer of streptomycin. The formation of streptomycin by an active strain takes place alike in stationary and in submerged culture. In the practical production of streptomycin, the formation of inactive variants by *S. griseus* need not be feared; conditions for obtaining the inactive variants are so completely different from those commonly employed in the laboratory or in the industrial production of the antibiotic agent that the possibility of strain degeneration is very small indeed. However, the use of vegetative growth for inoculation purposes may occasionally result in inactive lots.

ISOLATION OF STREPTOMYCIN

Streptomycin is water soluble and is insoluble in such organic solvents as ether, chloroform, and acetone. It may be readily isolated from the medium and concentrated by a method similar to that previously developed for streptothricin (2, 11). When the culture broth has attained its maximum antibiotic activity, incubation is terminated. The mass of growth of the organism is separated from the broth by centrifugation or by filtration. The selection of a filter is important, since streptomycin is rapidly adsorbed on various filters. The cell-free culture fluid, alkaline at the time of recovery, is treated with active charcoal, such as Norit, which adsorbs the antibiotic completely. The charcoal adsorbate is centrifuged or filtered off, and washed first with alcohol to remove some of the impurities and then with dilute acid-alcohol to bring the streptomycin into solution. After the removal of the charcoal, the acid-alcoholic solution is neutralized and filtered to remove certain impurities that have precipitated out. The resulting solution is then concentrated by the addition of 10 volumes of ether, which takes up the alcohol and leaves the streptomycin as a yellow-, brown-, or red-colored aqueous concentrate. A solid preparation may be obtained by precipitation of such a concentrate with acetone or by desic-

cation *in vacuo*. Much of the early work on the toxicity and antibacterial action of streptomycin *in vivo* was carried out with impure material prepared in this manner.

Further purification, and finally crystallization of streptomycin involve the preliminary preparation of an impure concentrate obtained by the above-mentioned adsorption on charcoal and its subsequent elution with dilute acid solution. After precipitation with phosphotungstic acid, the liberated bases are converted into the crude picrate which is then fractionated by chromatographic techniques. Upon removal of the picric acid, one or several of the resulting fractions yield amorphous products which are water soluble and highly active. The addition of Reinecke salt, $NH_4[Cr(SCN)_4(NH_3)_2]$, to aqueous solutions of these active fractions gives crystalline precipitates. From this mixture, pure streptomycin reineckate can be isolated by fractional crystallization. The insoluble reineckate can be converted to soluble salts, such as the hydrochloride or the sulfate (34).

A second method for the crystallization of streptomycin also utilizes the acidic eluate of the charcoal adsorbate. The addition of methyl orange (the sodium salt of helianthine) results in crystallization of the relatively insoluble streptomycin-helianthate, which can then be converted to soluble salts of streptomycin (36). To obtain the hydrochloride, the helianthate is treated with a mixture of methyl alcohol and hydrochloric acid. After removal of the liberated helianthine, the hydrochloride is precipitated by the addition of ether. The final product is a white powder.

METHODS OF ASSAY

In order to test for the presence or absence of streptomycin in a culture medium, a qualitative procedure for determining inhibition of growth of certain test bacteria is satisfactory. However, for the production and isolation of this antibiotic, as well as for pharmacological investigations and clinical use, it is essential to use quantitative methods to measure antibiotic potency. For this purpose, several methods are commonly used, namely, the liquid dilution method, the agar dilution streak method, the agar cup or agar diffusion method, the turbidimetric method, or some other convenient procedure.

In the agar dilution streak test, several bacteria are used, namely, *E. coli*, *S. aureus*, *B. subtilis*, and *B. mycoides*. This is a simple and rapid procedure, both for the qualitative demonstration of the streptomycin and for its approximate quantitative estimation (44). By using a number of test organisms, it is possible to obtain a bacteriostatic or antibacterial spectrum which is characteristic for this antibiotic. This is important not only for the purpose of establishing the specific nature of the streptomycin, but also to distinguish it from streptothricin and from other substances that can easily be isolated from different actinomycetes, which may be similar to streptomycin although not identical with it.

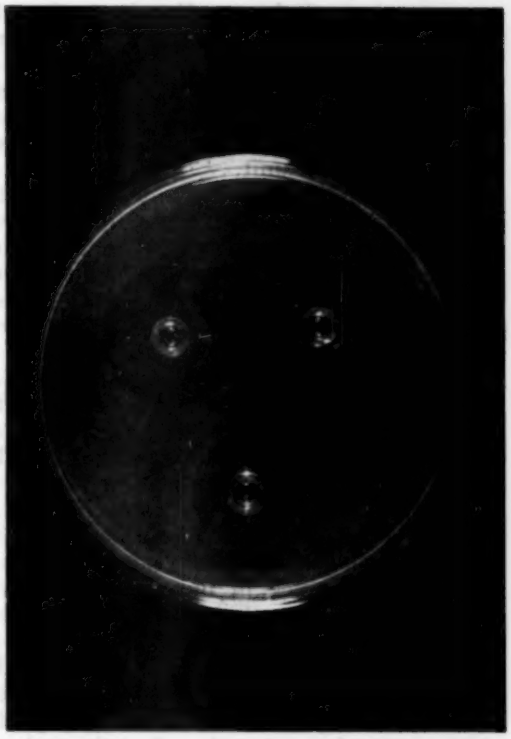

Fig. 2.—Antibacterial action as illustrated by the gradual diffusion of an antibiotic substance in a bacterial agar plate. Effect of streptomycin on *B. subtilis* (49).

For more accurate quantitative measurements, the agar diffusion or cup method may be used, with either *S. aureus* or *B. subtilis* as the test organism (Fig. 2). The activity of unknown preparations is compared with the inhibition of growth produced by a given standard, and the potency is calculated from the comparative values obtained and charted on a graph. Originally, a unit of streptomycin, or an S unit, was arbitrarily defined as

that amount of material which, when present in 1 ml. of nutrient medium, will just inhibit the growth of a given strain of *E. coli* (37). This unit is a convenient quantitative standard for the study of the production and isolation of streptomycin, as well as for microbiological studies of its antibacterial action *in vitro*. The small size of this unit, however, is rather awkward for pharmacological investigations and clinical use. Consequently, there were proposed, in addition to the S unit, an L unit, or that amount of material that will just inhibit growth of the particular strain of *E. coli* in 1 L. of nutrient medium, and a G unit based on the activity of the crystalline material. For the pure streptomycin base, a G unit was found to approximate closely 1,000 L units or 1,000,000 S units. Since crystalline streptomycin is now available, a new standard is being established based on the weight of the pure material, one microgram of which is equivalent to one unit.

It is of utmost importance that the unit of streptomycin be clearly understood. This is especially true when attempts are made to compare the efficacy of streptomycin with that of penicillin, since the two antibiotics are standardized differently. Whereas the Oxford unit of penicillin is based on inhibition of the Gram-positive *S. aureus* in 50 ml. of medium, the streptomycin unit is defined in terms of its action against the Gram-negative *E. coli* in 1 ml. of medium. The Oxford unit represents a much greater *in vitro* activity than does the streptomycin unit. Therefore, any comparison between the two agents which does not appreciate this fundamental difference in unitage may very likely confuse rather than clarify experimental results. In terms of dry weight, 1 streptomycin unit equals 1 γ, whereas 1 Oxford unit of penicillin equals 0.6 γ. It is to be remembered, however, that the activity of streptomycin is measured against the rather resistant Gram-negative bacteria, such as *E. coli*, and that of penicillin against the more sensitive Gram-positive bacteria, such as *S. aureus*. Actually, the Gram-positive *B. subtilis* is nearly 10 times more sensitive to streptomycin than *E. coli*, whereas the latter is many times more resistant to penicillin than *S. aureus*.

For the determination of streptomycin in body fluids, a modification of the agar cup method may be used (38). Since normal blood contains a diffusible agent which produces large zones with *B. subtilis* as the test organism, a particular strain of *S. aureus* is employed instead. The medium contains a low salt concentration and is adjusted to *p*H 7.5–8.0.[1] For titrating streptomycin in blood, dilutions of the unknown serum in normal serum are cup-tested in the usual manner. The standard is prepared to contain 1, 2, 4, 8, and 16 γ of streptomycin per ml. of normal serum. For feces, urine, or tissue extracts, distilled water replaces normal serum as the diluent.

Streptomycin can also be assayed, either as a solution of the concentrate prepared in phosphate buffer (*p*H 7.9) or in the active culture broth, by the use of a paper disc-plate method (52). This technique is essentially an agar-diffusion method and consists of pouring agar, inoculated with *B. subtilis* spores, upon the surface of plates already containing solidified sterile agar as a base. The medium used for this purpose is Bacto-Streptomycin Assay Agar, Dehydrated,[2] with a final *p*H of 7.9 ± 0.1. The plates are stored at 2–4° C as soon as poured and keep satisfactorily for several days. Dry filter paper discs are placed on the agar and 0.08 ml. portions of the streptomycin solutions are added as rapidly as possible (within five seconds). After 15 hours at 30°C. or four to six hours at 37°C. the zones of inhibition are measured. Using eight discs for each solution tested, a standard error of 8.5% could be expected.

In pharmacological and clinical work, streptomycin can also be quantitatively determined by the slide cell method similar to that used for assaying penicillin in body fluids; the principal change is the substitution of *Bacillus megatherium* for *Streptococcus pyogenes* as the test organism (30). A comparison of this modified slide cell method with the agar cup method, in which the medium was adjusted to *p*H 8.0 and *B. subtilis* used as the test organism, indicated that the agar cup technique can detect smaller concentrations of streptomycin. The latter method is also superior for the assay of streptomycin in urine. On the other hand, the slide cell gave satisfactory results for assaying streptomycin in blood, spinal fluid,

[1] The final composition of this medium is:

Peptone	10.0 Gm.
Beef extract	5.0 Gm.
NaCl	2.5 Gm.
Agar	10.0 Gm.
Distilled water	1000 ml.

Adjust to *p*H 7.5–8.0 with *N*/1 NaOH.

[2]

Peptone	6.0 Gm
Beef extract	1.5 Gm
Yeast extract	3.0 Gm
Agar	15.0 Gm
Distilled water	1000.0 ml.
*p*H after sterilization	7.9 ± 0.1

bile, and other fluids. With the slide cell method, sulfonamides, at the commonly encountered therapeutic levels, do not interfere in the determination of streptomycin in blood. Within certain limits it is also possible to assay streptomycin in the presence of penicillin, and vice versa.

In testing for sterility of streptomycin preparations or for the purpose of determining the presence of other antibiotics admixed with streptomycin, it may become necessary to inactivate streptomycin. Inactivation by cysteine (35) offers a possible procedure, especially as cysteine has no effect upon streptothricin. Streptomycin is also inactivated by 2-aminoethanethiol and to a lesser degree by thioglycollic acid. Neutral solutions of the organic sulfur compounds are added to known amounts of streptomycin or streptothricin dissolved in neutral phosphate buffer and, after several hours, tests are made for antibiotic potency, using the cup method with *B. subtilis* as the test organism. By the use of cysteine, one can not only differentiate the two antibiotics but also determine the relative amounts of each in mixtures of the two, as shown in Table IV. The inactivation of streptomycin by cysteine can be reversed by iodine. The regeneration of the streptomycin is carried out by shaking the solutions with small amounts of a carbon tetrachloride solution of iodine until no further decolorization occurs. The solutions are then aerated to remove the organic solvent. The recovery of the streptomycin activity is quantitative. The inactivation of the streptomycin is reversible, and is not a property of the sulfhydryl group alone, nor is it limited to cysteine.

CHEMICAL AND BIOLOGICAL PROPERTIES OF STREPTOMYCIN

Because of its solubility in water, insolubility in organic solvents, adsorption on charcoal from an alkaline solution, and elution by dilute acids, as well as the high nitrogen content of the concentrated preparations, streptomycin was from the beginning suspected of being an organic base. This initial assumption was proved correct when the material was finally crystallized (Fig. 3).

Anhydrous streptomycin helianthate darkens at 205°, and melts with decomposition at 220–226°. When the helianthate is converted to the hydrochloride, a white powder is obtained which, when dry, assays 800 units per mg. The hydrochloride is characterized by a specific rotation of $[\alpha]_D = -84°$, and the absence of sulfur and phosphorus in the molecule. In borate buffer at *p*H 9.0, in phosphate buffer at *p*H 7.0, and in glycine buffer at *p*H 2.0, the ultraviolet absorption spectrum of streptomycin revealed only end absorption below about 2300 Å. The sulfate was reported by one group of investigators (36) to give 520 units per mg. Other workers (34), however, found 850 units per mg. for streptomycin sulfate, and 500–530 units per mg. for streptothricin sulfate.

TABLE IV.—INFLUENCE OF CYSTEINE CONCENTRATION UPON THE ACTIVITY OF STREPTOMYCIN (46)

Mg. Cysteine per 100 γ of Streptomycin	Streptomycin Activity, Units, after 0 Hr.	5 Hr.	24 Hr.
0.0	37.5	37.5	25.5
0.5	37.5	29.5	22.0
2.5	23.0	14.0	2.0
12.5	2.0	2.0	1
25.0[a]	1.7	1.7	1
50.0[a]	5.3	4.5	1.8

[a] Results obtained with 25.0 and 50.0 mg. cysteine were rather confusing; several zones of alternating inhibition and stimulation were produced. The cysteine itself at similar concentrations appeared to have a marked inhibiting effect on *B. subtilis* by the cup assay method.

Streptomycin reineckate crystallizes in the form of thin plates from water (34), and decomposes at 162–164° C. (corr.). Inconsistencies in the analytical results of the anhydrous salt prevented the assignment of a definite empirical formula. Nevertheless, the formulas $(C_{14}H_{26}O_7N_9S_4Cr)n$ and $(C_{14}H_{26}O_8N_9S_4Cr)n$, which correspond to $(C_{10}H_{19}O_{7-8}N_3)n$ for the basic component, have been considered to be in reasonable agreement with the available data.

In contrast to penicillin, streptomycin is markedly stable both chemically and biologically. Solid preparations of relatively high activity have been stored in the refrigerator for periods greater than six months without any loss in potency. Dilute sterile solutions after fifteen to seventeen days at 37° C., have maintained their initial activity. Heating solutions of streptomycin for ten minutes at 100° C. produces an inactivation which is less than 50 per cent. Crude as well as highly concentrated solid preparations are hygroscopic.

Streptomycin is not destroyed by microorganisms. It is not labile in this respect as are some other antibiotics such as penicillin, gliotoxin, and pyocyanase. When streptomycin solutions, prepared, on the one hand, with water, and on the other, with nutrient

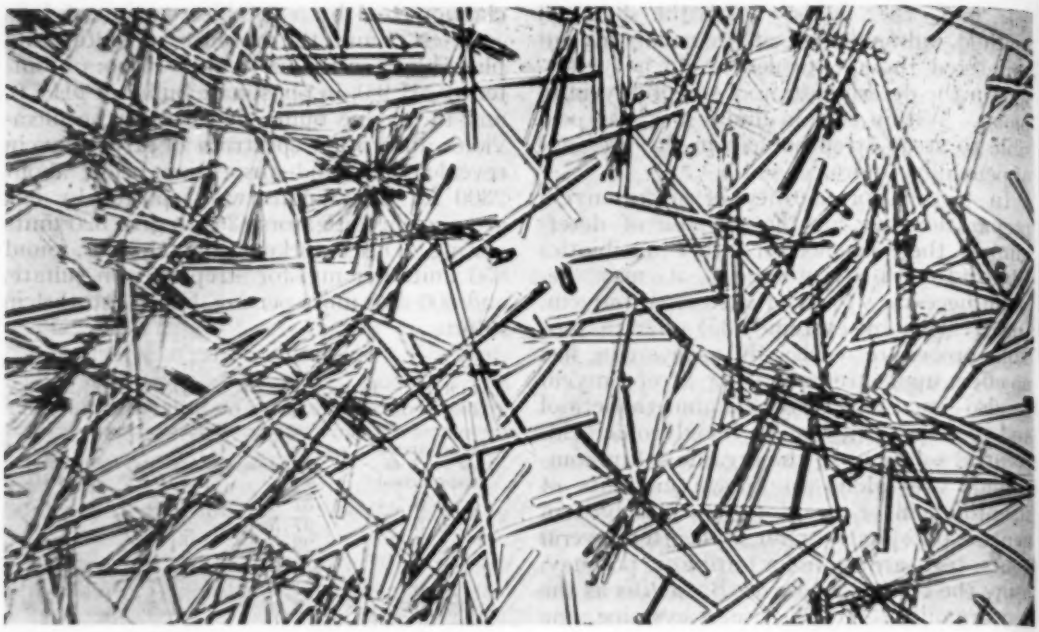

Fig. 3.—Crystalline streptomycin, double salt. Courtesy of Folkers, *et al.*, Merck and Company (51).

broth, were inoculated with *Aspergillus niger* or a mixed culture of bacteria and fungi, the activity of the cultures, after incubation at 28° C. for seven days, was no less than that of the sterile controls.

ANTIBACTERIAL ACTION OF STREPTOMYCIN *IN VITRO*

The action of streptomycin is generally bacteriostatic in low concentrations and bactericidal in larger amounts. Although streptomycin possesses a lytic effect upon the living cells of *B. subtilis*, it does not act upon the dead cells of this organism or upon living *S. aureus*. It is active against a variety of pathogenic Gram-positive and Gram-negative bacteria, including *M. tuberculosis*, *Eberthella typhosa*, *Pasteurella tularensis*, *Klebsiella pneumoniae*, *Brucella abortus*, *Pr. vulgaris*, *Salmonella schottmülleri*, *Hemophilus pertussis*, *H. influenzae*, *Ps. aeruginosa*, and many others. Streptomycin has only a limited effect *in vitro* upon spore-forming anaerobes. However, experimental infections caused by *S. aureus* and *Diplococcus pneumoniae* have been readily controlled with adequate doses. It also exerted a definite bactericidal effect *in vitro* upon a mixed culture of an anaerobic *Streptococcus* and *Pr. vulgaris*.

Streptomycin is active against acid-fast bacteria. *M. phlei* and the pathogenic H37 strain of *M. tuberculosis*, var. *hominis* were found to be almost as sensitive as *B. subtilis* and *B. mycoides;* however, *M. avium* was more resistant than either *E. coli* or *Ps. aeruginosa*. In order to kill the tubercule bacillus, an exposure of the cells to 200-300 units per ml. for a few days or to smaller amounts for longer period of time was necessary. Streptomycin has a marked effect upon organisms related to the mycobacteria. *Erysipelothrix muriseptica* and various pathogenic and saprophytic actinomycetes were inhibited by rather low concentrations of streptomycin. However, the active strains of *S. griseus*, the organism which produces streptomycin, are exceedingly resistant to this antibiotic. Streptomycin has very little activity against fungi.

Streptomycin is not adsorbed upon the cells of the bacteria; Gram-negative organisms subjected to its action revealed an elongation of the cells (22). Since the morphological changes were induced at greater dilutions than the minimal inhibitive level, it appears that the antibiotic agent may "influence the normal rate of division and hence the pathogenicity of the organism."

The antibiotic action of streptomycin depends upon the composition of the me-

TABLE V.—EFFECT OF *p*H AND GLUCOSE ON THE ACTIVITY OF STREPTOMYCIN[a] (19)

Preparation *p*H of Medium	Streptomycin Reaction Effect
9.0	2500
8.0	2000
7.0	1550
6.0	700
5.0	400
Glucose[b]	**Glucose Effect**
0	1200
2	600
10	620

[a] Activity in units of a given preparation.
[b] Mg. per plate.

dium, its reaction and presence or absence of certain substances. Among these, the salt effect, reaction of the medium, and the presence of glucose are particularly significant. The last two effects are illustrated in Table V. The optimum *p*H for the activity of streptomycin is 9.0. The reaction effect may be due to the fact that streptomycin, being a base, is not so active in the form of a salt as in a free basic state, or that the active part of the molecule is neutralized by the acid. The presence of glucose tends to reduce the activity of streptomycin appreciably, 2 mg. of glucose in 10 ml. agar being sufficient to reduce by one-half the potency of streptomycin. This may be due to the reducing properties of the glucose or to the production of some acid by the test organism. It is to be recalled that streptothricin shows the same sensitivity to salts (phosphate), to acidity of medium, and to glucose (7, 8).

Not only is streptomycin strongly bacteriostatic against various Gram-positive and Gram-negative bacteria, but it also exerts a marked bactericidal effect, as brought out in Table VI. This experiment illustrates the effect of streptomycin upon two strains of *Pr. vulgaris* and upon a resistant strain (R) developed by gradual passage through media containing the antibiotic. In the absence of streptomycin, rapid multiplication occurred in all three cultures. The addition of 1 unit or 1 γ of streptomycin reduced the numbers of strain W1 from 375 million to 10 thousand per ml. and of W2 from 615 million to 650 thousand; the reduction of the resistant strain, however, was only from 560 to 190 million per ml. The addition of 5 units or γ brought about nearly complete destruction of all the cells of cultures W1 and W2, whereas the reduction of the resistant culture was only two-thirds of the original number of cells. There is no doubt that even the resistant culture was subject to the bactericidal action of the streptomycin, but much more of the antibiotic was required to bring this about.

TABLE VI.—BACTERICIDAL ACTION OF STREPTOMYCIN UPON *Pr. vulgaris*[a] (40)

Streptomycin, γ per 1 Ml.	*Pr. vulgaris*, W1	*Pr. vulgaris*, W2	*Pr. vulgaris*, Strain R
	At start		
	39.5	45.5	26.0
	After 28 hours' incubation		
0	375.0	615.0	560.0
1	0.01	0.65	190.0
5	0[b]	0[b, c]	9.0
25	0	0[b, c]	3.13

[a] No. of cells in millions per 1 ml.
[b] 1 colony on a plate from 1:1000 dilution.
[c] 7–8 colonies on a plate from 1:10 dilution.

The problem of variation in sensitivity of different strains of the same organism to the action of streptomycin is of considerable importance from a chemotherapeutic point of view (40). The sensitivity of a given culture of an organism to streptomycin is characteristic not only of the organism but of the strain as well. This is brought out in

TABLE VII.—VARIATION IN SENSITIVITY OF DIFFERENT STRAINS OF THREE BACTERIA TO STREPTOMYCIN (40)

E. coli Culture No.	D. U.[a]	*Pr. vulgaris* Culture No.	D. U.	*S. aureus* Culture No.	D. U.
ATCC 26	30	ATCC 6898	30	ATCC 152	25
ATCC 6522	30	ATCC 7829	75	ATCC 6518	25
ATCC 6880	10	ATCC 8259	50	ATCC 6538	45
ATCC 6881	30	ATCC 8427	10	ATCC 8094	30
ATCC 8677	100	ATCC 9484	30	ATCC 8431	20
ATCC 8739	30	W1	30	W1	30
W2	30	W2	20	W2	25
W4	30	Av.	35	W4	75
W5	30			Merck Institute	30
Av.	35			Av.	34

[a] D. U. = dilution units per 1 mg. of the streptomycin preparation used, as determined by plate method. 1 D. U. = 1 γ of streptomycin for standard strain of *E. coli*.

Table VII, where the activity of a crude preparation of streptomycin against a number of strains of three organisms is reported. These results show that the sensitivity of an organism to streptomycin is more characteristic of the strain than of the species.

PHARMACOLOGY, TOXICOLOGY, AND EFFECTIVENESS OF STREPTOMYCIN IN ANIMALS

Studies of the absorption and excretion of streptomycin in mice, dogs, and monkeys have shown that this antibiotic behaves by parenteral administration like penicillin in that both agents are rapidly absorbed and excreted. Therapeutic blood levels are easily produced by intravenous or subcutaneous injections. Oral dosage, on the other hand, results in very low blood concentrations. Subcutaneous injections of 100–200 γ protect mice against lethal infections with *S. schottmülleri*, whereas by oral administration much more streptomycin is required.

TABLE VIII.—TOXICITY OF STREPTOMYCIN AND STREPTOTHRICIN (22)

Streptomycin	No. of Mice	γ/20 Gm.	% Dead
Low potency	20	1,250	0
Low potency	25	2,500	28
Low potency	20	5,000	90
High potency	15	5,000	0
High potency	15	7,500	0
High potency	15	10,000	0
High potency	10	15,000	90

The toxicity of streptomycin, especially of purified preparations, was found to be extremely low (Table VIII). When present, it revealed itself in animals usually as a histamine-like reaction, the visible symptoms in mice being increased activity, marked dyspnea, and, occasionally, respiratory failure (22). In rabbits, the more toxic lots caused a decrease in blood pressure, an effect similar to that produced by streptothricin. In view of the fact that the unfavorable reactions are not observed with many impure batches and are absent when pure preparations are used, this type of toxicity is undoubtedly due to certain impurities present in the toxic lots. In contrast to streptothricin, with which death is delayed for a few days or longer, any lethal action of streptomycin is revealed within twenty-four hours or less after administration.

TABLE IX.—ACTIVITY OF STREPTOMYCIN IN MICE INFECTED WITH *S. schottmülleri*[a] (18)

No. of Mice	Treatment	Dilution of Bacterial Culture Used for Infection	Survival of Mice in Hours		
			18	30	72
5	Control[b]	10^{-5}	2	0	0
3	Control	10^{-6}	1	0	0
3	Control	10^{-7}	1	0	0
3	Control	10^{-8}	2	0	0
5	Streptomycin, 6.4 mg.[c]	10^{-5}	5	5	5
5	Streptomycin, 12.8 mg.[c]	10^{-5}	5	5	4

[a] 30 γ streptomycin per 1 mg. of preparation.
[b] No streptomycin.
[c] Divided into 4 doses, at 6-hr. intervals.

According to Molitor and Mushett (48), of the Merck Institute, a second toxic effect, especially pronounced in monkeys, is observed following prolonged administration with large doses of streptomycin. This reaction occurs as a fatty infiltration of the liver and occasionally of the kidney. The metamorphosis involves the presence of fine to moderately sized fat globules primarily in the cord cells of the liver. In the kidney, the fat deposition is always slight, being primarily in the basal portion of the renal epithelium. This type of toxicity is ordinarily not found in rats, and appears to be related to the low protein diet of the monkeys. The organ pathology of the monkeys has been shown to be a reversible phenomenon and not the preliminary step of a progressive degeneration.

The first animal studies with streptomycin were carried out with crude preparations assaying about 30 γ per mg; their toxicity was LDO = 35 mg. and LD 100 = 135 mg. per 20 Gm. mouse, by intraperitoneal injection. To protect 18–20 Gm. mice infected with *S. schottmülleri*, 6.4 mg. of the crude material was required (Table IX). With *Ps. aeruginosa*, n ice were protected by

TABLE X.—ACTIVITY OF STREPTOMYCIN IN CHICK EMBRYOS INFECTED WITH FOWL TYPHOID[a] (18)

No. of Eggs	Treatment	Dilution of Bacterial Culture	Survival of Embryos in Days				Died from Fowl Typhoid
			2	4	7	12	
3	Control	10^{-6}	3	1	0	0	3
3	Control	10^{-5}	3	0	0	0	3
5	Streptomycin, 5 mg.	10^{-6}	4	3	2	2	1
5	Streptomycin, 5 mg.	10^{-5}	5	5	4	3	0
5	Streptomycin, 10 mg.	10^{-5}	5	4	3	0	0

[a] 30 γ streptomycin per 1 mg. of preparation.

TABLE XI.—EFFECT OF STREPTOMYCIN ON *Brucella abortus* IN CHICK EMBRYOS[a, b] (18)

Total No. of Embryos	Treatment	Presence of *Brucella abortus* in Embryos[c] Membrane	Liver
5	Control, untreated	4	3
6	Streptomycin, 5 mg.	2	0
6	Streptomycin, 10 mg.	0	0
6	Streptomycin, 20 mg.	0	0

[a] 4000 cells of *Br. abortus* injected into each chick embryo.
[b] 30 γ streptomycin per 1 mg. of preparation.
[c] The tests for the presence of *Br. abortus* were made on the death of the embryo or on the twenty-first day of incubation of the eggs.

190–380 units or by 6.4–12.8 mg. Complete protection of experimental animals infected with *Pr. vulgaris* was also obtained with streptomycin. The results of studies with 11-day-old chick embryos have shown that adequate concentrations of streptomycin can control fowl typhoid caused by *Shigella gallinarum* (Table X). Excellent protection was also obtained against *Brucella abortus* in chick embryos (Table XI). Infections due to certain Gram-positive organisms have been found to be somewhat more resistant to streptomycin. Nevertheless, experimental infections caused by *Diplococcus pneumoniae* and *S. aureus* were readily controlled by adequate doses of streptomycin. Streptothricin, under the same conditions, was not effective.

Streptomycin was found to have a marked protective effect in experimental tularemia in mice (24) (Table XII). In an experiment where the mortality of 30 control animals was 100 per cent within ninety-six hours after infection, the survival of 30 mice that received 1000 γ of streptomycin daily was 100 per cent for ten days. Those animals that had initially received 10 lethal doses of the organism possessed slight immunity to 1000 lethal doses twenty-eight days after the original inoculation. In view of the high morbidity of this disease and the lack of successful serum therapy, the favorable effect of streptomycin suggested that the antibiotic might find use in the treatment of tularemia in human beings.

In experimental infections with *Klebsiella pneumoniae*, streptomycin also exerted a marked protective effect (Table XIII). With doses of 185–500 γ daily, administered over a period of two to three days, 42 out of 49 mice survived intra-abdominal inoculation of 1000 to 10,000 lethal doses of the pathogen; in contrast to this, all 49 untreated controls died. Somewhat similar results were obtained with intranasally infected animals. Though treatment with 500 γ daily for three days gave only slight protection, an extended treatment for seven days enabled the animals to survive in 13 out of 15 cases, the mortality of the 15 controls being 100 per cent. These results suggested the possible usefulness of streptomycin in the treatment of human infections

TABLE XII.—EFFECT OF STREPTOMYCIN ON MICE INOCULATED WITH *Pasteurella tularensis* (24)

	Mice That Died Days after Inoculation 3	4	5 to 8	to 23	No. of Mice That Died	Mortality Rate, %
Treated with 1000 γ per day (30 mice)	0	0	0	0	0	0
Treated with 500 γ per day (12 mice)	0	0	4	1	5[a]	42
Untreated (30 mice)	13	17	0	0	30	100

[a] Two other mice died of pneumonia on the twenty-sixth and thirty-second days, respectively.

TABLE XIII.—EFFECT OF STREPTOMYCIN ON EXPERIMENTAL INFECTIONS CAUSED BY THE FRIEDLÄNDER GROUP OF BACTERIA (25)

Experiment	Infecting Dose: No. of Times Lethal Dose	How Given	Treatment with Streptomycin[a]: γ/Day	No. of Days	Mice Inoculated	Mice Died	Mortality, %
1	10,000	Intra-abdominally	185	3	8	0	0
			460	2	6	0	0
			None		14	14	100
2	1,000	Intra-abdominally	500	3	20	1	5
			250	3	20	6	30
			None		20	20	100
3	1,000	Intra-abdominally	500	3	15	0	0
			None		15	15	100
4	100	Intranasally	500	3	10	8	80
			None		10	10	100
5	100	Intranasally	500	7	15	2	13
			None		15	15	100

[a] The streptomycin was administered in divided doses as described in the text.

due to Friedländer bacillus. Streptomycin inhibits the growth of *H. pertussis* in concentrations of 3 γ per ml. and is bactericidal in concentrations of 15 γ. When mice were infected with 5000 cells and 2 mg. streptomycin given for ten days, 50 per cent of the mice were protected and the survival time of those that died was extended to twenty-two days as compared to five and one-half days for the controls (45).

The development of the human strain of *M. tuberculosis* in guinea pigs can be controlled by streptomycin (26), as brought out in Table XIV. In experimental infections of guinea pigs with *M. tuberculosis, hominis*, carried out at the Mayo Clinic by Drs. Feldman, Hinshaw, and Mann, streptomycin has been found to exert a strikingly suppressive effect upon the pathogen under the conditions imposed (29). On the basis of an arbitrarily established index of infection, microscopically determined, 100 units represented the maximum possible amount of tuberculosis. The control animals, sacrificed after sixty-one days, exhibited an index of 67 as contrasted to 5.8 for those which had received streptomycin. In another experiment, the corresponding values were 81.9 for the untreated and 2.8 for the treated animals. The daily administration of streptomycin varied from 1387 to 6000 γ. Two different strains of the human tubercle bacillus were equally sensitive *in vivo*.

TABLE XIV.—EFFECT OF STREPTOMYCIN UPON THE DEVELOPMENT OF HUMAN TUBERCULOSIS IN GUINEA PIGS (26)

Experiment	Animals	Duration of Infection, Days	Duration of Treatment, Days	Index of Infection Determined Microscopically[a]
1	1	43[b]	0	81.9
Controls	1	53[b]	0	
(8 animals)	2	54	0	
	4	60	0	
1	2	54	39	2.8
Treated	2	54	54	
(4 animals)				
2	9	61	0	67
Controls				
(9 animals[a])				
2	4	61	47	5.8
Treated	5	61	61	
(9 animals[a])				

[a] Of the ten animals in the group originally, one died prematurely.
[b] These animals died; other animals were killed.

The ability of streptomycin to check and retard the normal course of tuberculosis infection in guinea pigs has been confirmed. By combining streptomycin and promin, it was possible to obtain results unlike anything heretofore obtained in the treatment of experimental tuberculosis infections (53).

The disease in the control animals exhibited the usual destruction and wide dissemination. On the other hand, the treated animals manifested barely detectable evidence of tuberculosis. In most cases, the disease had remained localized at the site of inoculation and at the contiguous axillary lymph nodes. Microscopic examination of the controls revealed the typical, progressive, morbid processes of the lungs, liver, and spleen. The animals receiving streptomycin presented an entirely different picture: no lesions or only a very few grossly visible lesions. In many cases, the disease was absent or was minimal and arrested or nonprogressive. In one experiment, 9 out of 23 animals still living at the end of the period of treatment exhibited a reversal of sensitivity to tuberculin. For 7 of these 9 animals negative to tuberculin, the spleens were noninfective.

Although it appears that streptomycin may prove to be the most effective agent yet found for the treatment of experimental tuberculosis in guinea pigs, it should be emphasized that these findings must be viewed with the utmost conservatism. The very resistant nature of tuberculosis, the lack of uniform correlation between animal experiments and behavior of an agent in human beings, and the fact that some drugs of the sulfone series have given results comparable to those obtained with streptomycin are only some of the reasons why a very cautious attitude must be assumed toward the practical use of this antibiotic in tuberculosis. Streptomycin also exerted a marked protective effect in experimental infections with *Borrelia novyi* and *Leptospira icterohaemorrhagiae* (28), as shown in Table XV, but it did not suppress malarial infections (27).

THE TOXICOLOGY AND PHARMACOLOGY OF STREPTOMYCIN IN MAN

According to Molitor, Robinson, and Mushett (48) of the Merck Institute, both the toxicological and pharmacological properties of streptomycin readily allow its use in the treatment of diseases in man. Pure streptomycin, as well as many impure concentrated preparations, has been given at therapeutic levels, in doses as high as 4 Gm. daily, without any unfavorable reactions.

TABLE XV.—COMPARATIVE EFFECTS OF STREPTOMYCIN AND PENICILLIN ON *Leptospira icterohaemorrhagiae* IN HAMSTERS (28)

Units[a] per Day	Treatment Antibiotic	No. of Hamsters	Hamsters That Died Each Day after Inoculation: 7 to 10	11 to 14	15 to 22	No. of Hamsters That Died	Mortality Rate, %
250	Streptomycin	6	1	5	1	6	100
	Penicillin	6	..	..	..	6	100
500	Streptomycin	6	2	2	..	4	67
	Penicillin	6	..	..	..	0	0
800	Streptomycin	6	..	..	..	0	0
	Penicillin	6	..	..	..	0	0
	None	12	12	..	..	12	100

[a] For streptomycin, 1 unit equals 1 γ; for penicillin 1 unit is an Oxford unit or 0.6 γ of crystalline material.

In order to produce toxic manifestations in animals, it is necessary to administer either extraordinarily large amounts of the pure material or smaller amounts of certain impure preparations containing a histamine-like substance. No correlation was obtained between the acute toxicity of different lots to experimental animals and clinical tolerance by human beings. The behavior of the material in man cannot, therefore, be foretold if antibacterial potency alone is assumed to be the criterion of purity; highly concentrated material has on occasion been unsatisfactory clinically, whereas considerably less active preparations have often produced no undesirable effects. This lack of correlation is believed to be due primarily to the fact that the lethal and morbid toxicities of streptomycin do not result from the same causes and that only the latter is observed in man. The problem is further complicated by the difficulty of studying many reactions, such as drug fever and arthralgia, in experimental animals, the variation of individual patient susceptibility, and the inability of many preparations to produce clinical side-reactions even when administered in excessively large amounts.

Two distinctly different types of toxicity are possible. One, which is observed only with certain impure preparations, is due to a histamine-like substance. The general syndrome produced in man consists essentially of nausea, throbbing headache, general malaise, skin rash, pain in the joints, flushed face, vomiting, and drug fever, the last appearing two or three days after treatment and therefore not due to bacterial pyrogen. Local reactions at the sites of injection have also been encountered. These toxic manifestations are entirely absent when crystalline streptomycin as well as many impure preparations are used. By employing only pure streptomycin or impure concentrates which do not possess this factor, it is possible to eliminate the undesirable side-reactions.

The second kind of toxicity, which results in a fatty metamorphosis of the liver and kidney of animals, is an inherent property of streptomycin itself. However, this organ pathology has not been observed in man. Clinical information obtained by Hinshaw (48), of the Mayo Clinic, in studies with patients who had received 1 to 4 Gm. of pure streptomycin daily for twenty to ninety days has failed to demonstrate any nephrotoxic properties of streptomycin; nor was there any functional impairment of the liver which could be attributed to prolonged administration of the agent. Hinshaw and Feldman (50) reported the results of preliminary impressions obtained from the study of 34 patients who had tuberculosis and were treated with streptomycin for a period of nine months. It appeared that streptomycin exerted a limited suppressive effect, especially on some of the more unusual types of pulmonary and extrapulmonary tuberculosis. However, although the reproduction of *Mycobacterium tuberculosis* appeared to be temporarily inhibited by the treatment, no convincing evidence was obtained as to a rapidly effective bactericidal action.

Streptomycin has been administered intramuscularly, intravenously, and subcutaneously both by intermittent injection and by continuous drip. It has also been given orally and by nebulization into the tracheobronchial tree. By the intravenous route a high serum concentration is produced very rapidly, whereas the same level is obtained but more slowly after intramuscular or subcutaneous injection. However, the blood level falls rapidly following a single dose and must be maintained by repeated administrations. When given orally or by nebulization into the tracheobronchial tree, no appreciable amount of

streptomycin is absorbed into the general circulation.

According to Adcock (48), of the University of Michigan, the intravenous injection of single doses of 200 mg. of streptomycin gives an immediate concentration in the serum of 32 γ per ml. This falls rapidly to 8 γ at the end of two hours and to 1 or 2 γ at the end of twelve hours. Intramuscular injection of the same amount gives a peak serum level of 16 γ at the end of one hour, followed by a slower drop in serum concentration, with detectable amounts after twelve hours. Streptomycin rapidly appears in the urine, 20 to 35 per cent being recovered in two hours, and 50 to 70 per cent in the twelve-hour period after administration of a single dose. The total recovery varied from 15 to 85 per cent of the daily dose, with an average of 53 per cent. When given parenterally, small amounts of streptomycin appear in the spinal fluid, namely, 1 to 2 γ as compared to 25 γ in the serum. Following oral administration, very little streptomycin reaches the serum, and only 0.2 to 0.5 per cent is found in the urine; however, there is a high concentration in the feces.

Similar results were obtained by Zintel (48), of the University of Pennsylvania, and by others. An intravenous injection of 600 mg. gave 32.8 γ per ml. of blood in fifteen minutes with a decrease to 4.9 γ in six hours. Following a similar intramuscular injection, the rise is slower, with 26.5 γ at three hours and 8 γ at six hours per ml. of blood. The recovery of the streptomycin in the urine varied during the first twenty-four hours, between 29 and 89 per cent, with an average of 66 per cent.

Reimann (31, 48), of the Jefferson Hospital, has also found that parenteral administration of streptomycin leads to its rapid excretion through the kidneys. As much as 35 per cent has been recovered in the urine within two hours after injection, and up to 75 per cent and more of the daily amount administered. After a single dose, streptomycin has been detected in the urine for as long as thirty-six hours. On the other hand, the oral administration of 4 Gm. daily resulted in only a trace in the blood and urine. When ingested, therefore, streptomycin is not appreciably absorbed nor is it destroyed in the intestine. This persistence and stability in the gut has been used to advantage for the treatment of typhoid patients. The oral administration of steptomycin rendered the feces completely negative for *E. typhosa* and considerably reduced the numbers of *E. coli* as well as the general fecal population.

The studies of Hirshfeld (48) at Wayne University, together with those of Zintel (48) have shown that when the blood concentration is high, some streptomycin does diffuse into the cerebrospinal fluid, but the amount is generally slight. On the basis of the available information, it would seem that intrathecal injection is a much more satisfactory means for producing therapeutic levels in the spinal fluid. After parenteral administration, streptomycin has been detected in the ascitic, peritoneal, and pleural fluids, in concentrations approximating the level in the blood. Lesser amounts have been found in the bile. According to Adcock (48), a trace to several micrograms was present at autopsy in the heart muscle, brain, and liver of two treated patients.

CHEMOTHERAPY

Although considerable experimental work is being conducted on the clinical use of streptomycin, only a limited amount of the unintegrated information is available at the present time. To date, streptomycin has been tried in human infections resistant to penicillin, the sulfa drugs, and serum therapy. According to Greey (48), of the University of Toronto, in the treatment of chronic infections of the urinary tract, streptomycin is effective in destroying such Gram-negative bacteria as *Pr. vulgaris*, *A. aerogenes*, *E. coli*, *Ps. aeruginosa*, and *Eberthella* sp. Four hours after commencement of streptomycin therapy (1 Gm. of streptomycin daily in 8 divided doses given intramuscularly), urinary cultures were negative for *Pr. vulgaris* and, after eight hours, for coliform organisms. In one case, the urine became negative for *E. coli* two hours after treatment. Though infections of the normal urinary tract were permanently cleared up, reinfection was likely to occur in damaged tracts, the catheter serving as the portal of entry for the new infection. Similar results have been obtained by the U. S. Army Medical Corps in the successful treatment of heretofore resistant urinary tract infections.

Streptomycin has also proved effective for the treatment of enteric and systemic diseases. In five severe to moderately severe cases of typhoid, studied by Reimann (31), streptomycin was not administered until late in the development of the disease. Nevertheless, the clinical improvement of

three patients coincided with the period of streptomycin therapy. Of the two unsuccessful cases, the treatment of one was prematurely discontinued because of the limited quantity of streptomycin available; failure in the other has been postulated as a result of inadequate dosage or the presence in the body of a substance inhibitive to the action of streptomycin. The latter explanation is not very plausible, however, in view of the severity of the particular case, the long delay in parenteral administration, and the fact that no specific streptomycin inhibitor has yet been demonstrated in the human or animal body. Although the different strains of typhoid bacteria varied in their sensitivity to streptomycin, there was no evidence of increased resistance developed *in vivo* during the period of therapy. Although oral administration alone was inadequate to produce appreciable blood levels and urine concentrations essential for typhoid control, it nevertheless rendered the feces free of *E. typhosa*. In general, it has been suggested that for the treatment of bacillary infections of the intestinal and urinary tracts, streptomycin be given both orally and parenterally, the former during the disease as well as in the convalescent period to prevent reinfection and the carrier state. Oral administration also has been suggested under certain conditions as a prophylactic measure against intestinal infections.

In systemic infections, streptomycin has been used by Reimann (48), to combat *Brucella* infections. In view of the variable behavior and chronic nature of the disease and the limited number of patients thus far treated, no conclusion concerning the efficacy of streptomycin against *Brucella* infections is justifiable at the present time. The results, though encouraging, are inconclusive.

In *Klebsiella* infections, streptomycin has been found to exert a much more definite action. When treated by Herrell, of the Mayo Clinic [quoted by Heilman (25)], two patients with Friedländer infections of the respiratory tract, showed prompt disappearance of *Klebsiella* upon the institution of streptomycin therapy. Previously, the pathogen had been persistently present in the sputum.

Flippin (48), of the University of Pennsylvania, observed that *Salmonella* as well as *E. coli* infections lend themselves readily to treatment with streptomycin. A patient with a colony count of 23 million *Salmonella* in the stool gave a negative stool after four days oral therapy with 1 Gm. streptomycin daily; the number of *E. coli* was reduced simultaneously to about 1000; *Strep. faecalis* disappeared and the clostridia were reduced from 75,000 to 8000. Acute brucellosis was successfully treated by intramuscular administration of streptomycin.

According to Foshay (48) of the University of Cincinnati, *P. tularensis* is one of the most sensitive organisms *in vitro* to the bactericidal action of streptomycin. With only a few γ per ml. the killing effect is complete within a matter of seconds or of minutes at the most. It is not surprising, therefore, that the parenteral administration of relatively low doses of streptomycin has proved remarkably successful in human tularemia. Although the mortality of this disease is low, its morbidity is high; for over 500 untreated cases, the mean duration was 3.9 months. One patient who began to receive streptomycin on the eighth day of the disease was sent home as cured on the seventeenth day. In another case with perisplenitis and generalized infection of the peritoneal cavity, the peritoneal fluid was noninfective on the sixth day after treatment, whereas usually such fluid is infective for at least nine months. Of seven cases which had received streptomycin all responded promptly.

Hinshaw and Feldman (47) at the Mayo Clinic treated 22 tubercular patients with streptomycin without any serious toxic effects, even after prolonged administration of large doses. They concluded, however, that any decision as to the therapeutic efficiency of the antibiotic must await further study. Hinshaw (48) later observed, in a variety of human infections of *M. tuberculosis*, that treatment with streptomycin gave some encouraging results.

It must be emphasized that the information available is much too limited in scope for any evaluation at the present time. The pathological nature of the disease and the clinical characteristics are such that prolonged treatment and studies of many cases are absolute prerequisites for any serious consideration of the efficacy of streptomycin in the treatment of tuberculosis. To date, sufficient information has not been accumulated.

Limited results have been obtained for meningitis from the treatment of a dozen or so cases. According to Margaret Smith (48), of Sydenham Hospital, Baltimore, and Birmingham (48), of the Johns Hopkins

TABLE XVI.—RANGE IN SENSITIVITY OF DIFFERENT BACTERIA AND ACTINOMYCETES TO THE BACTERIOSTATIC ACTION OF STREPTOMYCIN[a]

Organism	γ	References
Aerobacter aerogenes	0.5–2.5	(17), (22)
Bacillus anthracis	0.375	Heilman (48)
B. cereus	0.83	(17)
B. megatherium	0.25–3.0	(17), Heilman (48)
B. mesentericus	1.67	(17)
B. mycoides	0.1–3.8	(17), (22)
B. subtilis	0.12–1.0	(17), (40)
Brucella abortus	0.5–3.75	Reimann (48), Heilman (48)
Br. melitensis	0.5	Slavin (48)
Br. suis	0.5	Reimann (48)
Clostridium butylicum	8.34	(17)
Cl. septicum	>105	(22)
Cl. sordellii	>105	(22)
Cl. tetani	>104	(22)
Cl. welchii	>104	(22)
Corynebacterium diphtheriae	0.375–3.75	Heilman (48)
Diplococcus pneumoniae	8.0	(22)
Eberthella typhi	1.0–37.5	(22), Heilman (48)
Erysipelothrix muriseptica	2.5	(20)
Escherichia coli	0.3–3.75	(40), Heilman (48)
E. communior	1.0–4.0	(40)
Hemophilus influenzae	1.56–5.0	Birmingham (48)
H. pertussis	1.25–3.0	Heilman (48), (45)
Listerella monocytogenes	2.5	Heilman (48), (17)
Klebsiella ozenae	0.375–1.5	Heilman (48)
K. pneumoniae	0.625–8.0	(25)
Malleomyces mallei	10–>10.0	Heilman (48)
Mycobacterium avium	10.0	(20)
M. phlei	0.12	(20)
M. tuberculosis, var. *hominis*	0.15	(20), (16)
Neisseria gonorrhoeae	5.0	Heilman (48)
N. intracellularis	5.0	Heilman (48)
Pasteurella aviseptica	15.00	Heilman (48)
P. lepiseptica	0.5–2.5	(22), Heilman (48)
P. pestis	0.75–1.5	Heilman (48)
P. tularensis	0.15–0.3	Heilman (48)
Phytomonas pruni	0.25	(17)
Proteus vulgaris	0.4–3.0	(40)
Pseudomonas aeruginosa[b]	2.5–25.0	(17), (20)
Ps. fluorescens[b]	12.5	(17)
Sarcina lutea	0.25	(17)
Serratia marcescens	1.0	(17)
Salmonella aertrycke	4.0–10.0	(17), (22)
S. enteritidis	0.5	(22)
S. schottmülleri	2.0	(22)
Salmonella sp.	60.0	Birmingham (48)
Shigella paradysenteriae	0.25–3.75	(22), Heilman (48)
Staphylococcus aureus[c]	0.5–>16.0	(22)
Streptococcus faecalis	50.0	Greey (48)
S. hemolyticus	2.0–>16.0	(22)
S. lactis	4.0	(22)
S. salivarius	5.0–25.0	Greey (48)
S. viridans	>16	(22)
Vibrio comma	6.0–37.5	Heilman (48)
Actinomyces bovis	3.75	Heilman (48)
Nocardia asteroides	12.5	(20)
N. gypsoides	4.0–12.5	(20)
Streptomyces albus	0.4–1.25	(20)
S. antibioticus	<0.4	(20)
S. lavendulae	1.25	(20)
Streptomyces sp. 3462	4–12.5	(20)

[a] γ of streptomycin per ml. of suitable medium required to inhibit growth.
[b] Out of a group of *Ps. aeruginosa* and *Ps. fluorescens* (Hirshfeld, 48), some required over 256 γ/ml. to inhibit growth.
[c] Some staphylococci have been reported (Hirshfeld, 48) to require over 256 γ/ml. to inhibit growth.

Hospital, some patients recovered from influenzal meningitis following administration of streptomycin alone. When insufficient doses of the drug were used, there was

definite development of drug-fastness of the organism. It was believed that optimal treatment may finally comprise streptomycin coupled with sulfadiazine, or with serum therapy, or both. Sterilization of spinal fluid and blood was accomplished, in a case of a four-year-old boy, in nine hours, the treatment consisting of injections of 200 mg. streptomycin every two hours for five days. It was recommended that intrathecal administration of streptomycin should always accompany intramuscular therapy. One case of *Salmonella* meningitis was treated successfully with streptomycin, although it was suggested that the exact role of the antibiotic in this one patient should be considered as inconclusive. No cases of *E. coli* meningitis in newborns have yet been treated with streptomycin. Since such infections, which are not rare, are generally nonresponsive to the sulfonamides and are almost always fatal, streptomycin may prove effective. In the few patients with tubercular meningitis, the administration of streptomycin did not appear to help very much.

Howes (48), of Columbia University, studying the treatment of wound infections with streptomycin, observed that 100 γ per ml. did not affect the growth of tissue culture at all, whereas 200 γ was only moderately inhibitory. At least 14 wounds were sutured with a mixture of 200 γ of streptomycin and 5 per cent marfanil, without any infections of the wounds and no untoward reactions. The stability of streptomycin solutions, in contrast with those of penicillin, was believed to be of special significance.

Adequate surgery, followed by proper treatment with the antibiotic agent streptomycin will undoubtedly prove highly effective, as suggested by Hirshfeld (48) and others.

SUMMARY AND CONCLUSIONS

Streptomycin, an antibiotic agent produced by certain strains of *Streptomyces griseus*, is the result of an extensive search for an antibiotic agent that would be active against Gram-negative bacteria and mycobacteria, be nontoxic to animals, and be efficacious *in vivo*. It is produced in shaken or stationary cultures and may be easily assayed by the agar diffusion or cup, agar dilution or turbidimetric methods. Streptomycin is recovered from the medium by adsorption on charcoal and subsequent elution with dilute acid-alcohol. Concentrated preparations of streptomycin are further purified and finally crystallized as the helianthate or as the reineckate. Streptomycin is an organic base soluble in water but insoluble in different organic solvents. It is chemically stable and is resistant to microbiological destruction. It is not adsorbed on bacterial cells. The pure base possesses an activity of about one unit per microgram.

Streptomycin is extensively active *in vitro* against Gram-negative and Gram-positive bacteria, as shown in Table XVI. It is also active against organisms such as *Ps. aeruginosa*, *Serratia marcescens*, and *B. mycoides*, which are resistant to streptothricin. It is bacteriostatic in low concentrations and bactericidal in greater amounts. Bacterial strains resistant to streptomycin can be produced by growing the cultures in media containing streptomycin. However, the adapted variants may or may not be simultaneously resistant to other antibiotic agents. The natural sensitivity of an organism is characteristic of a particular strain rather than of a species, different strains varying greatly in this respect.

Streptomycin also exerts a marked bacteriostatic and bactericidal effect upon *Mycobacterium tuberculosis*, especially the human strains. It inhibits related organisms such as *Erysipelothrix muriseptica*, and pathogenic and saprophytic actinomycetes. It is not active against fungi.

The antibacterial action of streptomycin *in vitro* is inhibited by a low *p*H, by the presence of glucose, by cysteine and 2-aminoethanethiol, and to a limited degree by thioglycollic acid.

Animal inoculation studies have established that the toxicity of streptomycin is extremely low. Although some lots produce an unfavorable histamine-like effect, this

seems to be a matter of purification of the antibiotic, the available evidence indicating that the histamine-like factor is an impurity.

In vivo, streptomycin has successfully controlled experimental infections due to *Salmonella schottmülleri*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Shigella gallinarum*, *Brucella abortus*, *Klebsiella pneumoniae*, *Pasteurella tularensis*, and *Mycobacterium tuberculosis*. Against the Gram-positive *Diplococcus pneumoniae* and *Staphylococcus aureus*, protection was afforded by somewhat larger doses. Streptomycin has also protected against spirochaetal infections due to *Borrelia novyi* and *Leptospira icterohaemorrhagiae*. It is not effective in suppressing avian malaria or in the control of the few virus diseases tried.

In any comparison of the efficacy of streptomycin and penicillin, the very fundamental difference between the streptomycin unit (1 γ of pure base) and the Oxford unit (0.6 γ of crystalline penicillin) must be appreciated to avoid misleading conclusions.

In human beings, streptomycin is rapidly absorbed and excreted in the urine following parenteral administration. It is not absorbed appreciably from the intestine, nor is it destroyed there; in the gut it exerts a marked suppressive effect upon the microbial population.

Therapeutic blood and urine levels are readily established and maintained, by intramuscular or intravenous injection. By the oral route of administration, streptomycin does not readily enter the blood stream.

Following the oral or parenteral administration of streptomycin, no unfavorable effects have been encountered which could not be attributed largely to the presence of impurities in the preparations used.

To date, streptomycin has been administered for infections of the urinary tract, typhoid, brucellosis, *Klebsiella* infections, tularemia, tuberculosis, and wounds. Although the results in some cases have been very encouraging, in others, such as brucellosis and tuberculosis, no definite conclusion may as yet be drawn. In view of the limited clinical data, a final evaluation of the therapeutic potentialities of streptomycin is impossible at the present time.

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The Spectrophotometric Determination of Quinacrine Hydrochloride (Atabrine)*

By HAROLD BISSELL, H. W. MOELLER, and L. D. SEIF

Solutions of quinacrine hydrochloride have been found to have a minor absorption peak at a wave length of 424 mμ as reported by Carol and a much greater absorption at 280 mμ. Either wave length may be used for the quantitative determination. Details of the spectrophotometric determination and absorption-concentration curves at 280 mμ are presented.

THE absorption spectra of a great many medicinal compounds have been presented by Elvidge (1–4) and the quantitative determination of quinine by absorption spectrophotometry has been reported by Carol (5, 6). This method of quantitative measurement has also been applied to quinacrine hydrochloride (Atabrine) by Carol (7) using the visual and ultraviolet portions of the spectrum extending from 300 to 500 mμ. In the present paper, the region of observation has been extended further into the ultraviolet to 230 mμ. It has been found that there is a much greater degree of absorption in this lower wave-length region, particularly at 280 mμ.

EXPERIMENTAL

A Beckman quartz spectrophotometer (model DU) with a slit-width of 0.7 mm. and fused silica cells, 1 cm., was used in this work. It was calibrated on the basis of the 656.5 mμ hydrogen blue line and on a solution of potassium chromate in potassium hydroxide.

The reference material used was a standard sample of quinacrine hydrochloride (Atabrine), GT 172, furnished by Winthrop Chemical Co., Inc. This sample, on repeated examination, has been found to comply fully with the U. S. P. XII standards and with those established by the Surgeon General's Office, Washington, D. C. It has been found to contain 7.36% moisture and repeated analyses by the U. S. P. method (8) gave an average value of 98.0% quinacrine hydrochloride. This sample was used on the basis of the average U. S. P. assay rather than on a weight basis in order to correlate the spectrophotometric determination with the U. S. P. assay.

The absorption of the solution of separately weighed samples of the reference material was found to vary somewhat when equal amounts of acetic acid were added to the solutions. This variation was overcome by adding 20 cc. of a buffer solution, prepared by mixing equal parts of 0.5 *N* sodium hydroxide and *N* acetic acid, per liter of the original solution.

In Fig. 1 the spectral absorption curve is given for wave lengths from 230 to 500 mμ for a 0.02 Gm. per liter solution. The absorption above 300 mμ parallels that determined by Carol (7). Below 300 mμ the absorption increases very sharply and drastically, resulting in a high absorption peak at 280 mμ. The sharp peak at 280 mμ is a valuable index of the identity and purity of quinacrine hydrochloride. The absorption at 280 mμ may be used for the quantitative determination of the powder and tablets, and in Fig. 2 the absorption is plotted against concentration at this wave length. At 280 mμ the determination should be made with an instrument capable of using a spectral band much more narrow than that transmitted by a glass filter and in which the accuracy and duplication of the wave-length setting can be relied upon.

The spectrophotometric determination at 280 mμ has been applied equally well to pure quinacrine

* Received Jan. 29, 1945, from the Analytical and Control Department, Research Laboratories, The Wm. S. Merrell Company, Cincinnati, Ohio.

hydrochloride and to tablets. Ordinary tablet excipients, such as starch, talc and stearic acid, did not affect the absorption at 280 mμ or 424 mμ. Filtration of the tablet solution was found to be unnecessary in dilutions used for the determination. Tablet excipients, other than those mentioned, should be examined spectrophotometrically in the dilutions in which they occur in the determination in order to decide whether they would have any effect on the absorption. Hundreds of determinations on both powder and tablets have been made at 280 mμ. In many cases they have been checked against the U. S. P. methods (8–10) and, in each case, have been found to be more capable of close duplication than the official method.

Method.—About 1 Gm. of quinacrine hydrochloride, accurately weighed (or powdered tablet equivalent to this amount of quinacrine hydrochloride), is placed in a 1000-cc. volumetric flask. About 200 cc. of distilled water is added and the flask swirled until solution is effected. Forty cubic centimeters of buffer solution (equal volumes of 0.5 *N* sodium hydroxide and 1.0 *N* acetic acid) is added, the solution is diluted to the mark with distilled water and mixed thoroughly. A 5.0-cc. aliquot is diluted with distilled water to 1000 cc. in a volumetric flask and mixed well.

A sample from the final dilution is placed in a fused silica 1-cm. cell and the absorption determined at 280 mμ with water as a blank. Concentration is determined by reference to the absorption concentration curve.

The wave length of 424 mμ may also be used for the quantitative determination especially with a photoelectric colorimeter, using a filter with a very narrow spectral band, or with a visual absorption spectrophotometer. It was found that the determination at 424 mμ was reliable and would furnish an additional means of identification and check on the purity.

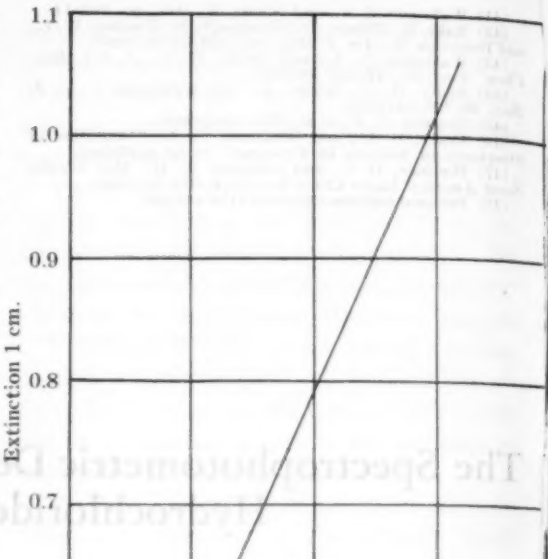

Figure 2.

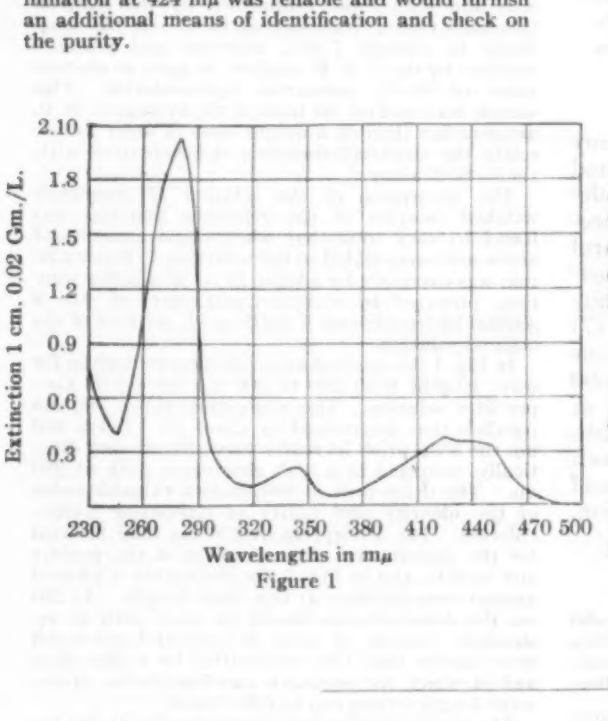

Figure 1

SUMMARY

The spectral absorption curve from 230 to 500 mμ of quinacrine hydrochloride solution has been plotted and a method for the quantitative estimation of quinacrine hydrochloride by means of its absorption at 280 mμ has been described.

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A New Nitroprusside Color Reaction for Bisulfite-binding Substances*

By RAYMOND JONNARD and MARVIN R. THOMPSON

A new color reaction for aldehydes, ketones, sugars, cystine and cysteine, and other substances capable of forming bisulfite addition compounds is described. This reaction explains the existence of abnormal positive Mörner, Legal, and Krishnaswamy reactions in protein hydrolyzates otherwise deprived of cystine and cysteine.

IN THE course of our preliminary studies on parenteral protein hydrolyzates (1) it was frequently observed that preparations reacting negatively by the Sullivan (2) procedure for the detection of cystine (with sodium-β-naphthoquinone sulfonate), nevertheless gave a positive Mörner reaction (with alkaline nitroprusside), either by the original technique (3), the Arnold (4) modification (using ammonia), or the Shinohara and Kilpatrick (5) procedure (in the presence of zinc acetate or sulfate).

In a number of these complex hydrolyzates the absence of cystine and cysteine could be demonstrated, notably by the mercaptide precipitation procedure described by Beveridge and Lucas (6). If the nitroprusside reaction was carried out in these hydrolyzates after a preliminary reduction by means of 20 per cent sodium sulfite in acid solution followed by a treatment with 5 per cent alkaline sodium or potassium cyanide as in Krishnaswamy's reaction (7), a rather stable pink color reminiscent of that due to cystine, still developed. Since it is well known that the Mörner reaction for cystine is lacking specificity, it was important to ascertain the nature of the color reaction reported.

The color obtained in hydrolyzates in the presence of sodium sulfite, as well as that developed in control tubes containing either pure cystine or cysteine and sulfite, or sulfite alone, was rather stable. This result is opposed to the transitory characters of the color resulting from the application of the original Mörner reaction in cysteine solutions. Since a somewhat similarly stable color developed also in the blanks (containing sulfite, or bisulfite, alone) of the Krishnaswamy's reaction, two hypotheses were formulated, namely: (*a*) that the time of contact allowed by the latter author (one to five minutes) was insufficient to ensure complete destruction of the excess of sulfite, and (*b*) that any substance capable of forming a bisulfite addition compound of the type RS—SO_3Na, or similar to that formed during the reduction of cystine to cysteine (Heffeter, 8), should give a color reaction provided conditions are found for the destruction of the uncombined sulfite without altering the composition of the addition compounds. This should be the case for a number of aldehydes and ketones, and for the sugars for which Browne (9) demonstrated the existence of bisulfite addition compounds

EXPERIMENTAL

Procedure.—The reaction was carried uniformly as follows:

To 1 mg. of the test substance dissolved (or suspended) in 5 ml. of water, add 1 ml. of 10% HCl (*v/v*) and 1 ml. of a freshly prepared 20% aqueous solution of sodium sulfite or bisulfite. Allow to stand at least fifteen minutes, for according to Browne some sugars reach equilibrium quite slowly. Then add 2 ml. of ammonia T. S. Verify that the *p*H of the mixture is approximately 7.5 to 9.0, avoiding too much alkali (the bisulfite ion ceases to exist at about *p*H 9.0). Add immediately 3 ml. of a 5% aqueous solution of sodium or potassium cyanide in 0.5 *N* NaOH, prepared at the time of use. Heat fifteen minutes in a boiling water bath, then cool under a stream of water. Divide the content of the tube into two portions. To one portion add 0.5 ml. of a fresh 5% solution of sodium nitroprusside. The color develops immediately. In the other portion, verify that all excess of sulfite or bisulfite is destroyed, by adding more cyanide solution (about 1 ml.) and reheating for five minutes before carrying out the test as above. The color should be similar to that developed in the first portion analyzed.

Controls containing the test substances listed in Table I, without sulfite and cyanide, are either colorless or develop transitory yellow or orange colors. Controls containing only the sulfite, or bisulfite, and cyanide without the aldehydes, are always yellow. Water-ammonia-reagent controls are colorless.

Table I condenses the results obtained with a number of test substances selected from the laboratory shelves. All the aldehydes and ketones tested gave a positive reaction, but the colors developed

* Received March 26, 1945, from The Warner Institute for Therapeutic Research, New York, N. Y.

by these two groups of substances are easily differentiated. All sugars with the exception of *d*-mannose gave a positive reaction.

DISCUSSION

The reaction described seems rather specific for the detection of substances capable of forming bisulfite addition compounds. It is likely that it will eventually lend itself to quantitative estimation when the spectrophotometric absorption curves of the colored derivatives reported are known.

solution, it permits the detection of β-hydroxybutyric acid in urine by Hart's procedure (17).

While the Legal reaction is interfered with by the presence of a number of sterols and of indolyl derivatives (Salkowski, 18 and Zipfel, 19), the proposed reaction results on the contrary in sharply contrasted colors with indole, skatole, and tryptophane.

The application of the new reaction to the analysis of protein hydrolyzates, conjointly with other cysteine and cystine reactions, avoids a great deal of confusion. From Table I it appears that no interference will result from the presence of either histidine or methionine. Both these amino acids,

TABLE I.—RESULTS OBTAINED WITH TEST SUBSTANCES

Test Substances	Color	Test Substances	Color
d-Ribose	Pink	Dextrose	Pink
Raffinose	Pink	Ca-hexosephosphate	Pink
Maltose	Pink	Pentacetylglucose	Yellow[a]
Trehalose	Pink	Ca-glucuronate	Brown[b]
d-Galactose	Pink	Ascorbic acid	Yellow
l-Xylose	Pink	Phytine	Yellow
Levulose	Pink	*d*-Mannose	Yellow
l-Sorbose (ketose)	Orange		
Formaldehyde	Pink	Furfural	Dark red
Glycerine-formal	Pink	Glycerine-furfural	Orange
Chloroethylformal	Yellow	Benzaldehyde	Orange
Haxanal	Dark red to green	*p*-Hydroxybenzaldehyde	Yellow
Heptaldehyde	Yellow	Vanilline	Orange
Acetone	Cherry-red	Anisaldehyde	Brown
Dicinnamyl-acetone	Orange	Xanthone	Yellow
Indole	Dark violet	Tryptophane	Yellow
Skatole	Yellow	Cholesterol	Yellow
Methionine	Yellow	3-Hr. casein hydrolyzate	Pink[d]
Histidine	Yellow	7-Hr. casein hydrolyzate	Pink[d]
Casein[c]	Pink	23-Hr. casein hydrolyzate	Pink[d]
		cystine and cysteine:	pink

[a] No specimen of aldehydo-pentacetyl-*d*-glucose (Wolfrom 24), in which a free aldehyde group gives a positive Schiff test, could be secured.

[b] Extracted from the hydrolytic products of gum acacia (unascertained purity).

[c] Carbohydrate-free, purified preparation described in a previous publication (25). The pink color is due here to the S—S group of cystine.

[d] These hydrolyzates do not contain any cystine nor cysteine: the color is due to the presence of aldehydes.

The results in Table I support the contention of Browne (9), based upon both rotation measurements and direct titration, that many sugars form bisulfite combinations contrary to the assertions found in a number of textbooks notably Pringsheim (10). The exceptional failure of *d*-mannose to react positively, as opposed to the behavior of its isomer dextrose, is also in line with Browne's data regarding the bisulfite addition reaction which is conditioned by the relative ease of opening of the pyranose ring, in the case of the sugars.

The nitroprusside-bisulfite reaction described presents a number of advantages over the Legal (11, 12) reaction for aldehydes (Meyer, 13) and ketones (Studer, 14), and over any of its more recent modifications such as that of Rantzman (15) who saturates the mixture with $AmNO_3$ in order to increase the sensibility. This new reaction can advantageously be applied in a similar way to that of the Harding and Ruttan (16) reaction for acetoacetic acid. After a preliminary distillation followed by oxidation with warm hydrogen peroxide

however, develop very similar colors by the Sullivan and McCarthy (20) reaction with nitroprusside in acid solution. The nitroprusside-bisulfite reaction thus becomes valuable for the detection of cystine, cysteine, aldehydes, ketones, and substances forming bisulfite addition compounds. This result becomes significant when it is recognized that aldehydes (acetaldehyde, pyruvic aldehyde, phenylacetaldehyde, etc.) are always present in acid and alkali protein hydrolyzates where they result from the autocatalytic oxidation of a number of amino acids in the presence of traces of oxygen and further hydrolysis of the Schiff bases formed, according to Wieland (21, 22), Herbst (23), and others. Such reactions take place even in the complete absence of carbohydrates. The presence of these aldehydes explains the aberrant results furnished in protein hydrolyzates by the often-recommended Mörner and Legal reactions and by their modifications mentioned earlier in these pages, and which now appear entirely inadequate for the analysis of these complex mixtures.

CONCLUSIONS

1. A new color reaction for aldehydes, ketones, sugars, cystine and cysteine, and other substances capable of forming bisulfite addition compounds, is described. This reaction explains the existence of abnormal positive Mörner, Legal, and Krishnaswamy reactions, in protein hydrolyzates otherwise deprived of cystine and cysteine.

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A Note on the Stability of the Iodine Content of Desiccated Thyroid*,†

By KENNETH L. WATERS‡ and GEORGE D. BEAL§

Desiccated thyroid powder and tablets stored for a period of ten years have shown no loss in iodine content.

THE stability of the iodine content of desiccated thyroid powder over a period of five years has been reported by Johnson and Nelson (1). These samples have been reanalyzed after a lapse of an additional five years. Four samples of thyroid tablets, the analysis of which was reported by Beal and Szalkowski (2) in 1935, have also been reanalyzed. The iodine content of the seven samples has not changed during the ten-year period. No special storage conditions were observed; the samples, contained in regular brown glass bottles closed with screw tops, have been held on laboratory shelves. Neither the powder nor the tablets showed any visible signs of deterioration; the tablets were firm and could be described as being commercially acceptable. A test for inorganic iodide (3) was negative, indicating that if any decomposition of thyroid iodide has taken place it has not been transformed into inorganic iodide.

The analyses of these samples are recorded in the Table.

TABLE I.—STABILITY OF THE IODINE CONTENT OF DESICCATED THYROID

Sample No.	Per Cent Iodine: Producer's Claim	1935 Analysis	1940 Analysis	1945 Analysis
1	0.218	0.236	0.234	0.233
4	...	0.202	0.193	0.199
5	...	0.202	0.200	0.200
Tablets				
2	0.0101	0.0099	...	0.0102
3	0.0820	0.0816	...	0.0824
14	...	0.0370	...	0.0375
7	0.0612	0.0612	...	0.0595

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The Detection of Water-Soluble Inorganic Iodide in Thyroid*,†

By KENNETH L. WATERS‡ and GEORGE D. BEAL§

A rapid qualitative test which may be used for the detection of adulteration of thyroid with water-soluble inorganic iodide is presented. The test consists of shaking the thyroid with saturated zinc sulfate, filtering, and adding sodium nitrite, dilute sulfuric acid and starch solution to the filtrate. A blue color develops with samples containing only 0.006 per cent inorganic iodine.

THE desirability of a pharmacopœial test to detect the adulteration of desiccated thyroid with inorganic iodide is obvious. The British Pharmacopœia, 1932 (1), included a determination of "inorganic iodide" based on the water-soluble iodine content of the desiccated thyroid. Rees and Salway (2) showed that this test is impractical in the case of glands dried at low temperatures since thyroglobulin is not denatured under such conditions and is water soluble. Harrington and Randall (3) confirmed the work of Rees and Salway (2) and admitted that the determination of "inorganic iodide" which they had proposed earlier (4) was not a measure of the inorganic iodide present. In view of these experiments as well as those of Rotter and Mecz (5) the British Pharmacopœia deleted the determination of inorganic iodide in the 1936 Addendum (6). The Canadian Supplement, 1944, to the British Pharmacopœia (7) describes a qualitative test for water-soluble inorganic iodide. This test is similar to one used by Fabre and Penau (8) to detect the adulteration of thyroid with iodized albumin. In our hands this test has not been particularly successful in the detection of added quantities of inorganic iodide.

We have developed a rapid qualitative test which will detect water-soluble inorganic iodine if present to the extent of one part in ten thousand parts of desiccated thyroid. With U. S. P. Thyroid the test will detect inorganic iodine if present in amounts as low as 3 per cent of the total iodine content. The test consists of an extraction of the inorganic iodide from the thyroid by shaking with a saturated solution of zinc sulfate. The iodide is detected in the filtrate after oxidation to free iodine with nitrous acid; the characteristic blue color of the starch-iodine complex is very intense with small amounts of free iodine. None of the sixteen commercial samples examined showed any trace of blue color when subjected to the test, whereas a sample containing only 0.006 per cent added inorganic iodine gave a distinctive coloration.

EXPERIMENTAL

The test which has been used successfully to detect the adulteration of thyroid with inorganic iodide is as follows:

Place 1 Gm. of desiccated thyroid or 3 Gm. of powdered tablets in a dry test tube and add 10 cc. of saturated zinc sulfate solution. Shake for about five minutes and filter through a fritted glass filter. To 5 cc. of the clear filtrate add 0.5 cc. of 0.5% starch solution, 4 drops of sodium nitrite (10%), and 4 drops of dilute sulfuric acid (10%), shaking after the addition of each reagent. The presence of inorganic iodide is indicated by the development of the characteristic blue color of the starch-iodine complex.

An adulterated sample of thyroid was prepared by adding a weighed quantity of finely powdered potassium iodide to a previously analyzed and weighed quantity of desiccated thyroid. The mixture was placed in a ball mill and milled for two hours. By calculation the increase in iodine content should have been 0.064%. However, analysis showed that the iodine content had been increased only 0.057%. To prepare additional adulterated thyroid samples a portion of the previously adulterated sample was mixed with an equal weight of the original desiccated thyroid and this mixture milled for two hours in a ball mill; this sample should contain one-half of the amount of inorganic iodine which was present in the first adulterated sample. A total of four adulterated samples were prepared in this manner. Table I gives the analysis of these samples.

TABLE I

Sample	Total Iodine, %	Added Inorg. Iodine, %	Inorganic Iodine as Per Cent of Total Iodine Content
I	0.260	0.057	21.9
II	0.230	0.027	11.7
III	0.215	0.012	5.6
IV	0.209	0.006	2.9
Original	0.203	None	None

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When the test described above was applied to these adulterated samples the intensity of the blue color was roughly proportional to the concentration of the added iodide. Sample I gave a very intense dark blue color whereas Sample IV gave a light blue, but still characteristic, color.

No blue color was obtained when the test was applied to seven samples of U. S. P. Thyroid Tablets and to eight samples of U. S. P. Thyroid Powder. A desiccated hog thyroid that analyzed 0.67% iodine also failed to show the presence of inorganic iodine. A blue color was produced when the test was applied to a mixture of diiodotyrosine and casein which had been standardized at 0.2% iodine by Johnson and Nelson (9) during the course of their work.

CONCLUSIONS

A rapid qualitative test for the detection of adulteration of thyroid with water-soluble inorganic iodide has been presented and shown to be applicable to U. S. P. thyroid powder and tablets.

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A Modification of the U. S. P. XII Assay for Desiccated Thyroid*,†

By KENNETH L. WATERS‡ and GEORGE D. BEAL§

A modification of the U. S. P. XII assay for desiccated thyroid is proposed. The procedure employs an ignition at 675–700° C. for twenty minutes with potassium carbonate followed by bromine oxidation. The results of a cooperative study with six laboratories are presented.

THE ideal assay for thyroid would be a determination of the physiologically active constituents. Unfortunately, biochemical evidence is not yet fully conclusive as to just what these constituents are, although the British Pharmacopœia (1) recognizes the importance of the thyroxine content of the glands. Until such conclusive evidence can be compiled to show that the concentrations of certain active principles of the thyroid gland are directly proportional to the physiological activity, the Pharmacopœia properly can only recognize an assay which is based on a determination of the total per cent of iodine. Many such assays have been reported in the literature. The most recent report is that of Englis and Knoepfelmacher (2) commenting on the cerate oxidimetric procedure of Hilty and Wilson (3).

Many of the methods have merit, but we have confined our study to established Pharmacopœia methods since it has been shown (4) that the U. S. P. XII (5) and the U. S. P. XI (6) assays for thyroid give results which are in very good agreement. Certain possibly valid objections may be made to both procedures. It was with the belief that an assay more satisfactory than either of the Pharmacopœia methods could be devised that we have continued our studies. Special attention has been given the time-saving features of both methods.

Among the objections to the U. S. P. XII procedure are wet mixing, the addition of shredded filter paper, and the preliminary drying period before ignition. An ignition mixture which does not require a drying period is convenient; such a mixture was employed in the U. S. P. XI assay. That mixture, however, was not without objection since the fused mass was sometimes difficult to dissolve. Johnson and Nelson (7), in the study on which the U. S. P. XII assay was

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based, showed that an oxidizing agent was not necessary in the determination of small amounts of iodine if large amounts of organic matter were present. We have found that the ignition of desiccated thyroid with potassium carbonate alone (omitting the addition of shredded filter paper) gives results which compare favorably with the results obtained using the U. S. P. XII assay. Such an ignition leaves a friable residue which is easily extracted with hot water; this avoids the fused mass of the U. S. P. XI and the bulky amorphous carbon of the U. S. P. XII methods.

TABLE I.—COMPARISON OF RESULTS OBTAINED ON DESICCATED THYROID POWDER BY THE U. S. P. XII ASSAY AND ITS MODIFICATION

Sample No.	Per Cent Iodine: U. S. P. XII	Modification
4	0.196	0.196
	0.196	0.196
11	0.675	0.674, 0.668
	0.666	0.673, 0.674
	0.668	
12	0.211	0.211, 0.211, 0.208
	0.213	0.206, 0.214
13	0.201	0.204
	0.205	0.203
	0.204	0.204
13 + 0.06% inorg. iodine added	0.258	0.260
	0.273	0.260
13 + 0.03% inorg. iodine added	0.231	0.232
	0.228	0.231
13 + 0.015% inorg. iodine added	0.214	0.216
	0.215	0.214
13 + 0.008% inorg. iodine added	0.210	0.212
	0.215	0.214

From a study of variations in temperature and time of ignition it has been concluded that these factors are not too critical; satisfactory results were obtained when the ignition was made at temperatures as high as 725° C. for a period of twenty-five minutes.

In an effort to eliminate entirely the returning blue color of the starch-iodine complex, indicating loss of the end point, so often encountered in both U. S. P. XI and U. S. P. XII thyroid assays, it was observed that this return would be just as readily obtained in a blank determination. After critical examination of the purity of all the reagents employed we have concluded that the returning color is due to the excess of bromine possibly forming some oxidized bromine derivative that does not dissociate readily and completely at low *p*H, and hence is not all removed as elementary bromine. This may cause a slow release of the iodine from the iodide, with return of the indicator color. If only a slight excess of bromine is added there is little or no return of the color.

The use of bromine water in the oxidation as described in the U. S. P. XII offers advantages over the chlorinated soda solution of the U. S. P. XI in that it is easily prepared, more rapid in its action, and just as complete in oxidizing power.

EXPERIMENTAL[1]

The modification of the U .S. P. XII assay for thyroid described below has given very satisfactory results as shown in Tables I and II.

Modification of U. S. P. XII Procedure.—Accurately weigh about 1 Gm. of thyroid or 2 to 4 Gm. of powdered thyroid Tablets into a porcelain crucible, and add 7 Gm. of anhydrous potassium carbonate. Mix thoroughly and gently tap the crucible on the desk several times to compact the mixture. Overlay with an additional 10 Gm. of potassium carbonate and compact the material thoroughly by tapping. Ignite the mixture for

TABLE II.—COMPARISON OF RESULTS OBTAINED ON THYROID TABLETS BY THE U. S. P. XII ASSAY AND ITS MODIFICATION

Tablet No.	Gr. per Tablet	Av. Wt. per Tablet, Gm.	Wt. Powder for Analysis, Gm.	Per Cent Iodine: Modification	U. S. P. XII
2	1/10	0.1676	3.0000	0.0104	0.0098
				0.0100	0.0106
7	1	0.2085	4.000	0.0596	0.0600
				0.0593	0.0594
9	1	0.1635	2.0000	0.0684	0.0694
				0.0688	0.0685
				0.0684	
				0.0699	
15	1	0.1941	3.000	0.0777	0.0777
				0.0777	0.0772
				0.0784	

[1] The authors are indebted to the following firms for furnishing thyroid samples and cooperating in the study of the modification of the assay: Burroughs Wellcome and Company, Sharp & Dohme, Inc., The Wilson Laboratories, The Zemmer Company, The Armour Laboratories, and The Wm. S. Merrell Company.

twenty-five minutes at 675–700° C. in a muffle furnace preheated to that temperature. Cool, add 20 cc. of distilled water, heat gently to boiling, and decant through an 18- to 19-cm. filter into a 500-cc. Erlenmeyer flask. Repeat the extraction by boiling with 20-cc. portions of dstilled water, then wash the crucible and the char on the filter with hot water until the filtrate measures approximately 200 cc. Add 7 cc. of Bromine T. S., then slowly add 38 cc. of phosphoric acid (1 in 2) and boil until starch-potassium iodide test paper is no longer colored blue by the vapors. Wash down the sides of the flask and continue to boil for about five minutes. Cool, add 5 cc. of phenol (1 in 20), again rinse the walls of the flask, and allow to stand for about five minutes. Add 2 cc. of phosphoric acid (1 in 2) and 5 cc. of potassium iodide T. S. and titrate immediately with 0.01 *N* sodium thiosulfate, adding 3 cc. of starch T. S. as indicator when the end point is neared. Perform a blank test with the same quantities of the same reagents and in the same manner, and make any necessary correction. Each cc. of 0.01 *N* sodium thiosulfate is equivalent to 0.0002116 Gm. of iodine.

Other modifications were also tried and found to be successful. One procedure which consisted of ignition with potassium carbonate as described above, leaching the ignition mixture as described, making the extract up to a definite volume, and taking an aliquot for analysis, was preferred to the above method by one of the cooperating laboratories. Another procedure proposed by Block and Waters (8) as applicable to pure organic iodine compounds also gave excellent results. Both of these assays require slightly more time than the one described above. In Table III the results obtained using the latter modifications are compared with those obtained by Johnson and Nelson (7) using the U. S. P. XI and XII method.

TABLE III.—COMPARISON OF IODINE DETERMINATION BY OTHER MODIFICATIONS

	Per Cent Iodine			
Sample	K_2CO_3 Ignition 200/250 cc. Aliquot Taken	Block and Waters Method (8)	U. S. P. XI[a]	U. S. P. XII[a]
1	0.225	0.232	0.237	0.237
	0.228	0.234		
4	0.195	0.200	0.191	0.196
	0.194	0.198		
5	0.199	0.199	0.199	0.200
	0.203	0.200		
DIT Mixt.[b]	0.185	0.185	0.189	0.189
	0.188	0.185		
DIT Mixt.[b]	0.213	0.212	0.210	0.212
	0.212	0.210		

[a] These results previously reported by Johnson and Nelson (7).

[b] Diiodotyrosine-casein mixture prepared by Johnson and Nelson (7).

Variation in Temperature and Time of Ignition.—The time and temperature of the ignition do not appear to be a critical factor. The U. S. P. XI ignites at a somewhat indefinite temperature for twenty minutes whereas the U. S. P. XII ignites at 525–550° C. for twenty minutes. Sappington, Halperin, and Salter (9) in a micro-iodine method for thyroid ignite at 600° C. for thirty minutes and then continue the heating for two hours at 550° C. We have found in a series of experiments that ignition with potassium carbonate at temperatures as high as 725° C. for twenty-five minutes gave the same results as were obtained when the U. S. P. XII method was used. Ignition with potassium carbonate at 650° C. for twenty minutes as compared with ignition for forty minutes also gave identical results.

TABLE IV.—COOPERATING LABORATORY REPORTS ON THE ASSAY OF THYROID

		Per Cent Iodine		
Laboratory	Lot No.	U. S. P. XI Assay	U. S. P. XII Assay	Modification of U. S. P. XII
A	..	...	0.190	0.199
B	..	0.207	0.188	0.198
		0.208	0.186	0.197
				0.195 Ni crucible
				0.195
C	65	0.1916	...	0.1702
		0.1936		0.1973
				0.1913
				0.2003
				0.1963
	72	0.1925	...	0.2010
		0.1933		0.2092
		0.1903		
		0.1892		
	69	0.1976	...	0.2093
		0.1960		
	70	0.2050	...	0.2175
		0.2008		0.2196
		0.2025		
		0.2015		
D		0.2304	0.2308	0.2412
Tablets calculated as grains thyroid per tablet				
E		1.03	...	0.257
F		...	1.14	1.15

Effect of Excess Bromine on the Returning End Point.—The return of the blue color of the starch-iodine complex in iodine titrations is a source of considerable annoyance. We have observed in bromine oxidation of iodide that a disappearing end point may be obtained on a blank consisting of potassium carbonate dissolved in distilled water. When 15 cc. of bromine water is used in the blank determination the end point disappears at such a rate that it requires the addition of 0.1 cc. of 0.01 *N* thiosulfate per minute to remove the returning blue color. However, if only 1 cc. of bromine water is added no blue color is observed to return to the solution even after standing.

In an effort to determine the cause of this end-point disappearance reagents were checked for purity and the bromine was purified by the method of Karns and Donaldson (10). The use of purified bromine produced no noticeable effect in the rate of the disappearance of the end point. As a routine practice the acidities of the titrated solutions were checked by means of a glass electrode; these were found to be approximately *p*H 2.5 in all cases.

It was observed that if the bromine water was added after the addition of the phosphoric acid there was no disappearance of the end point. Accordingly, a series of blank determinations were made in which the bromine water was added at various acidities. These showed that there was little or no disappearance of the end point if the bromine water was added at *p*H 4.5 or below. Unfortunately in the thyroid analyses in which the bromine water was added at *p*H 4.5 the results were about 25% low, showing either incomplete oxidation or loss of iodine.

In order to observe the effect of an excess of bromine water on the end point four blank determinations were made. These consisted of 13.5 Gm. of potassium carbonate dissolved in 200 cc. of distilled water to which were added 1 cc., 5 cc., 10 cc., and 15 cc., respectively, of saturated bromine water. The mixtures were then adjusted to *p*H 2.5 with phosphoric acid and the bromine removed according to the directions described in the modification of the U. S. P. XII method reported above. It was observed that no appreciable color developed in the mixture to which 1 cc. of bromine water had been added; however, in the other determinations the color started developing after about thirty seconds. Its intensity and rate of return was roughly proportional to the amount of excess bromine water added. We have concluded that, if the disappearing end point is to be eliminated, the excess bromine must be held to a minimum. Generally 7 cc. of bromine water has given satisfactory results.

Reports from Cooperating Laboratories on the Modification.—Three of the modifications of the U. S. P. XII assay which gave very satisfactory results in this laboratory were mimeographed and made available to other laboratories. Table IV summarizes the results obtained by the cooperating laboratories using the Pharmacopœia methods and the modification as described above; the samples analyzed are those of the individual laboratories and have not been analyzed by other laboratories.

Laboratories A and F preferred this modification to the Pharmacopœia assay; laboratory D stated that all methods were satisfactory but the modification described above was the most practical; laboratory B offered no objections but thought the U. S. P. XI procedure a better method; laboratory C stated that they did not like the U. S. P. XII method or any of the modifications thereof; laboratory E obtained satisfactory results on one of the modifications which employed the aliquot procedure, but were not satisfied with their results and are planning additional work.

CONCLUSIONS

A modification of the U. S. P. XII assay for thyroid has been presented and has been shown to give good results.

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The Microscopic Identification of the Crystalline Thiocyanates of Quinine, Quinidine, Cinchonine and Cinchonidine*

By GEORGE L. KEENAN and L. E. WARREN

Optical crystallographic data are reported for the thiocyanates of the four principal cinchona alkaloids which serve to differentiate quinine, quinidine, cinchonine, and cinchonidine.

In 1935 Monnet (1) published a method for separating quinidine from many mixtures. He found that quinidine thiocyanate is one of the least soluble salts of quinidine and that it is considerably less soluble than the thiocyanates of the other principal alkaloids of cinchona. The respective solubilities of these salts as found by Monnet are as follows:

TABLE I.—PROPERTIES OF THE THIOCYANATES OF THE PRINCIPAL CINCHONA ALKALOIDS

Thiocyanate	Solubility in Water at 20° C.
Quinidine	1477
Quinine	562
Cinchonidine	305
Cinchonine	474

In connection with the preparation of the thiocyanates of these four alkaloids, the senior author found that the crystalline precipitate formed in each case was adapted to microscopic study by the immersion method. All of these precipitates furnished optical crystallographic data which readily differentiated them. The method of preparation of these thiocyanates and the optical constants obtained are given in the following descriptions.

* Received July 6, 1945, as a joint contribution from the Microanalytical Division and the Drug Division of the Food and Drug Administration, Federal Security Agency, Washington, D. C.

EXPERIMENTAL

Quinine Thiocyanate.—This salt was prepared by precipitating a 10% aqueous solution of quinine hydrochloride with a 10% aqueous solution of ammonium thiocyanate and recrystallizing twice from water. The material crystallizes with 1 mole of water of crystallization. Analysis gave the formula as $C_{20}H_{24}O_2N_2 \cdot HCNS \cdot H_2O$. The salt is too soluble in water to have much value for the quantitative separation of quinine from the other alkaloids, but the reaction is of value in the detection of the alkaloid.

In ordinary light, the precipitate consisted of rods and needles, these frequently forming in circular aggregates when the ammonium thiocyanate reagent is added to a small amount of the alkaloid or its salt on a slide. In parallel polarized light (crossed nicols), the extinction is parallel and the sign of elongation is positive. Refractive indices: $n_\alpha = 1.604$, $n_\beta = 1.614$, $n_\gamma = 1.687$, all ± 0.002. n_α and n_β are commonly shown crosswise and n_γ lengthwise. Shaner and Willard (2) give the following refractive indices for quinine thiocyanate: $n_\beta = 1.61$ and $n_\gamma = 1.69$. No value is given for n_α

Quinidine Thiocyanate.—This salt was prepared by precipitating an aqueous solution of quinidine sulfate with an aqueous solution of ammonium thiocyanate. The salt was recrystallized once from 25% alcohol. Analysis indicated that the formula is essentially $C_{20}H_{24}O_2N_2 \cdot HCNS$. Another specimen was crystallized twice from water and this product contained no water of hydration.

In ordinary light the precipitate consisted of rods and needles closely resembling those characteristic of the thiocyanates of quinine, cinchonine, and cinchonidine. It also occurs in circular aggregates of rods and needles when the ammonium thiocyanate reagent is added to a small amount of the alkaloid or its salt on a slide. In parallel polarized light (crossed nicols), the extinction is parallel and the sign of elongation is negative. In convergent polarized light (crossed nicols), a partial biaxial interference figure showing one optic axis, occurs. Refractive indices: $n_\alpha = 1.549$, $n_\beta = 1.643$, $n_\gamma = >1.734$, all ± 0.002. n_β and n_γ commonly occur crosswise on rods, and n_α lengthwise. Many rods, however, match 1.734 crosswise.

Cinchonine Thiocyanate.—This salt was prepared by precipitating a solution of cinchonine sulfate with a solution of ammonium thiocyanate and recrystallizing twice from water. The salt contains no water of crystallization and the following formula was confirmed by analysis: $C_{19}H_{22}ON_2 \cdot HCNS$.

In ordinary light, this crystalline precipitate also consisted of rods and needles. In parallel polarized light (crossed nicols), the extinction is parallel and the sign of elongation negative. Refractive indices: $n_\alpha = 1.545$, $n_\beta = 1.650$, $n_\gamma = 1.705$ (± 0.003); n_α and $n_\beta \pm 0.002$. n_α is frequently shown lengthwise on rods and n_β and n_γ crosswise.

Poe and Swisher (3) have given the following refractive indices for cinchonine thiocyanate: $n_\alpha = 1.554$, $n_\beta = 1.651$, $n_\gamma = 1.700$. These values agree very well with those given above, the maximum difference being in the n_γ value, which is not significant from a determinative standpoint.

Cinchonidine Thiocyanate.—This compound was prepared by precipitating a 10% solution of cinchonidine sulfate by a 10% solution of ammonium thiocyanate. The resulting product was recrystallized twice from water. Analysis indicated that the formula for the salt is $C_{19}H_{22}ON_2 \cdot HCNS$.

In ordinary light the substance consisted of rods and needles, frequently forming in circular aggregates when precipitated by the addition of a 10% aqueous solution of ammonium thiocyanate to a small amount of the alkaloid on a slide. In parallel polarized light (crossed nicols), the extinction is parallel and the sign of elongation positive. Refractive indices: $n_\alpha = 1.600$, $n_\beta = 1.645$, both ± 0.002, $n_\gamma = 1.700$ (± 0.003). n_α and n_β are both shown crosswise on rods, n_γ being shown lengthwise.

A summary of the optical crystallographic data is given in the following table:

TABLE II.—OPTICAL DATA OF THE THIOCYANATES OF THE PRINCIPAL CINCHONA ALKALOIDS

Thiocyanate	n_α	n_β	n_γ	Extinction	Elongation	Habit
Quinine	1.604	1.614	1.687	Parallel	Positive	Rods
Quinidine	1.549	1.643	1.734>	Parallel	Negative	Rods
Cinchonine	1.545	1.650	1.705	Parallel	Negative	Rods
Cinchonidine	1.600	1.645	1.700	Parallel	Positive	Rods

TABLE III.—ANALYSES OF THIOCYANATES OF SEVERAL ALKALOIDS OF CINCHONA

	Loss on Drying			Alkaloid			Thiocyanic Acid (Theoretical)			
Thiocyanate	Found	Theory	Recovery	Found	Theory	Recovery	Found	Theory	Recovery	Probable Formula
Cinchonidine	0.14	0	0	83.4	83.3	99.9	16.50	16.72	98.6	$C_{19}H_{22}ON_2 \cdot HCNS$
Cinchonine	0	0	0	83.3	83.3	100.0	16.70	16.72	99.9	
				83.4		100.1	16.49		98.6	$C_{19}H_{22}ON_2 \cdot HCNS$
Quinidine	0	0	0	85.6		101.2				
				85.7	84.6	101.3	15.26	15.41	99.0	$C_{20}H_{24}O_2N_2 \cdot HCNS$
Quinine	4.84		107.7	80.7		99.95	14.04		95.4	$C_{20}H_{24}O_2N_2 \cdot HCNS$
	4.51	4.49	100.4	81.0	80.8	100.25	14.11	14.72	96.0	$+H_2O$
	4.54		101.3	...		...	...			

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A Comparative Study of the Suspending Properties of Clays with Special Reference to Bentonite*

By JOSEPH LA ROCCA† and HENRY M. BURLAGE‡

Evidence from physical and chemical data is presented showing that bentonite, due to its lattice-like structure more than its particle size, exhibits suspending properties superior to fuller's earth or kaolin. Stability of bentonite suspensions is shown to be greater in neutral or alkaline than in acid media. An identification test for bentonite is presented.

THE purpose of this investigation was to ascertain whether some clays, such as kaolin and fuller's earth, might have properties in common with bentonite. If so, the present monograph in the Twelfth Revision of the United States Pharmacopœia might be revised to include all of the clays now official.

An examination of literature on the subject (1) reveals that bentonite has a multitude of uses medicinally and commercially. It has been used in the manufacture of foundry molds, soaps, detergents, insecticides, fungicides, dips, scouring compounds, emulsions, paints, paper, inks, greases, ointments, face creams, tooth pastes, as a bleaching agent in the petroleum industry, in the clarification of wines, a water softener and coagulant, a clarifier-filtrant, a laxative, and a suspending agent. Its use as a pharmaceutical suspending agent was reported by Fantus (2), in 1933, who found that Calamine Lotion N. F., with 2.5 per cent bentonite, was more stable and stayed in suspension much longer than the official lotion.

Lewis, Squires, and Thompson (3) assume that the suspending properties of clays depend on an abundance of hydroxyl ions on the exterior surfaces of the clay crystal. These surface hydroxyl groups are amphoteric although their weakly acidic character predominates. As a result the highly polar hydroxyl groups exert strong fields of attraction in the immediate vicinity of the particle surface. Therefore, when adjacent surfaces come face to face, they are firmly held together, forming large, stable, linear aggregates resulting in a strong tendency toward gelatinization. These acidic clay surfaces are profoundly influenced by the *p*H of the suspending medium as will be shown experimentally in this study. The work of Lewis, *et al.* (4), has been substantiated by the writers who found that bentonite, when washed with acid, loses its suspending properties because the acid neutralizes the surface hydroxyl ions, thus preventing the formation of large, stable, linear aggregates.

COMPARISON OF THE COMPOSITION AND PROPERTIES OF BENTONITE, KAOLIN, AND FULLER'S EARTH

A. Official Tests.—Samples of these clays were subjected to the test for fineness of powder and identification of the twelfth revision of the Pharmacopœia (page 82). All passed the test for fineness of powder but only bentonite passed the identification test, indicating a definite relationship between the swelling property and the suspending action of this clay.

B. Chemical Composition.—An examination of the composition of these clays as reported by Ries (5) indicated that in so far as the major constituents—i.e., SiO_2 and Al_2O_3—are concerned, they are quite similar. The ratio of these two ingredients is 2.7 to 1, respectively, for bentonite, 1.5 to 1 in kaolin, and 2.8 to 1 in fuller's earth.

C. Crystalline Structure of Kaolin and Bentonite.—Marshall (6), by X-ray studies, has established the structure of the kaolin and bentonite aggregates and suggests that that of kaolin might apply to other clays which do not swell. Simple plane drawings of these structures are shown in Fig. 1. The

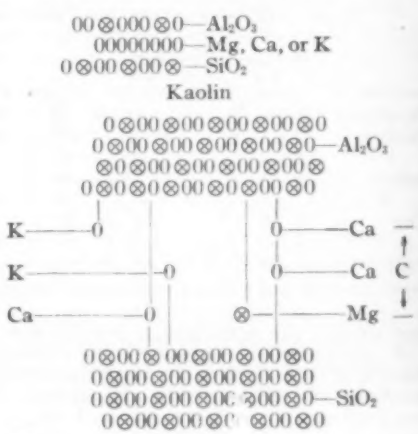

Fig. 1.

* Received Nov. 3, 1944, from the School of Pharmacy, University of North Carolina, Chapel Hill, N. C.

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‡ Professor of Pharmacy, University of North Carolina.

shape of the structure may be visualized by comparing it to a deck of playing cards in which every other card is a layer of Al_2O_3 molecules and the intervening cards are layers of SiO_2 molecules. In each case, each layer of Al_2O_3 is connected by the valence bonds of Ca, Mg, or K to a layer of SiO_2 molecules. However, the distances (C) between these layers for bentonite is variable with the amount of water present and in the case of kaolin the distances (C) are fairly constant with variable amounts of water.

D. Particle Size.—Hauser and Reed (7) find the particle size of bentonite to range from 14.3–87 mμ. and Marshall (6) finds those of kaolin and fuller's earth to be greater than 100 mμ. Bentonite, therefore, has the advantage over other clays of being applicable for pharmaceutical use after a simple screening process; it also differs from the other clays because of its swelling properties which, according to Reed (8), make it so valuable as a suspending and jelling agent. Furthermore, it possesses a physical structure (9) offering a large surface area which gives it unusual adsorptive properties.

EXPERIMENTAL

I. Identification Test of the Pharmacopœia.—Samples of the three clays (200 mesh) were subjected to the identification test of the Pharmacopœia (10). The volumes assumed by the particles averaged 15.3 cc., 3 cc., and 3 cc. for bentonite, kaolin, and fuller's earth, respectively. It was difficult to obtain an accurate reading of the volume for bentonite because of lumping and the failure to form a sediment that was level.

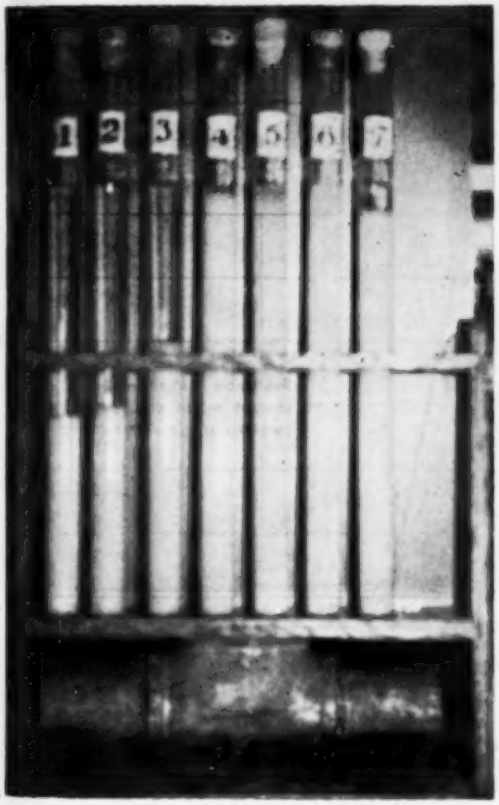

Fig. 2.—Bentonite Suspensions in Buffer Solutions after standing 332 hours.

II. The Effect of *p*H on Clay Suspensions (2% W/V).—Since the theory explaining the suspending properties of bentonite is based on the assumption that its molecule carries on its external surface an abundance of hydroxyl ions, it seemed desirable to study the suspensions of the three clays in buffer solutions of varying degrees of *p*H.

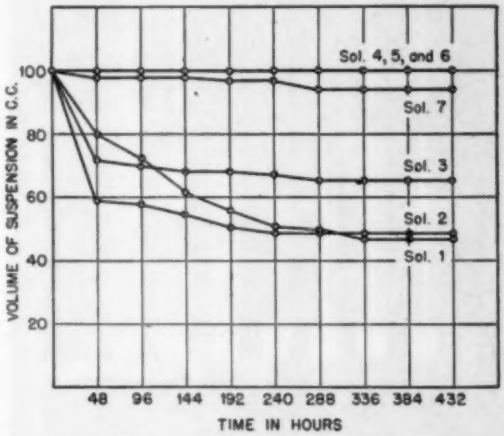

Fig. 3.—Stability of bentonite suspensions 2% in media of varied *p*H.

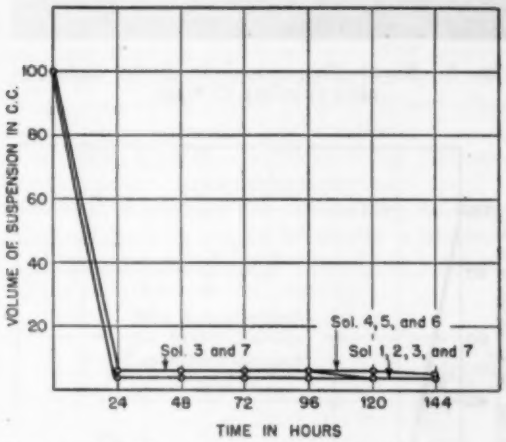

Fig. 4.—Stability of activated bentonite 2% suspensions in media of varied *p*H.

Therefore, buffer solutions as described in the Pharmacopœia (11) were prepared and their respective hydrogen ion concentrations determined by means of an electrometer with the following results:

Buffer Solution	U. S. P. Value	Electrometer Value
No. 1	1.00	1.10
No. 2	3.00	2.96
No. 3	5.00	4.90
No. 4	7.00	6.90
No. 5	8.00	7.80
No. 6	9.00	8.95
No. 7	10.00	9.75

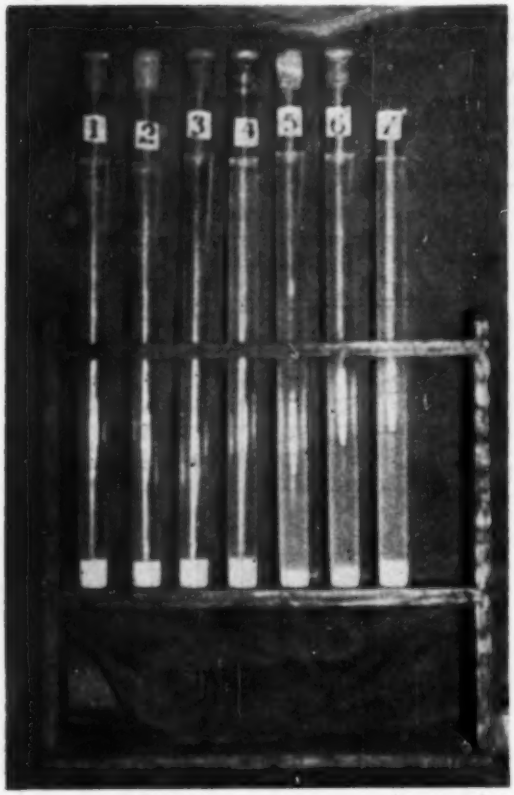

Fig. 5.—Kaolin Suspensions in Buffer Solutions after standing 72 hours.

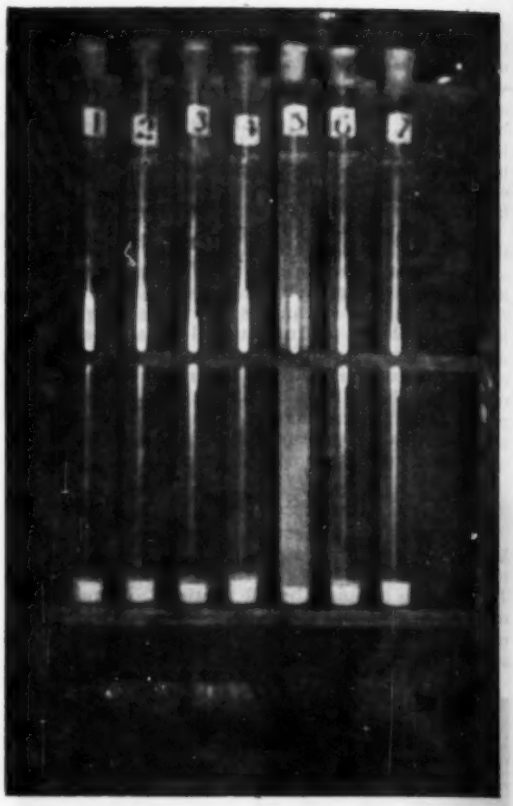

Fig. 7.—Fuller's Earth Suspensions in Buffer Solutions after standing 72 hours.

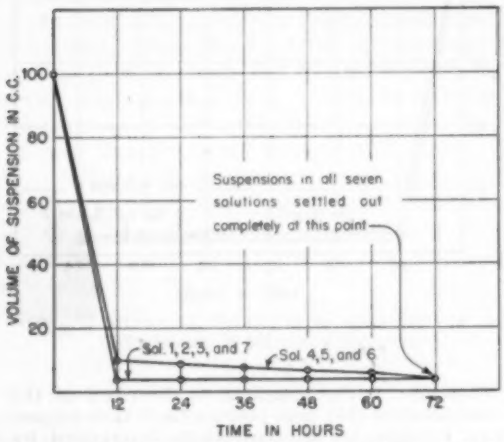

Fig. 6.—Stability of kaolin suspensions 2% in media of varied *p*H.

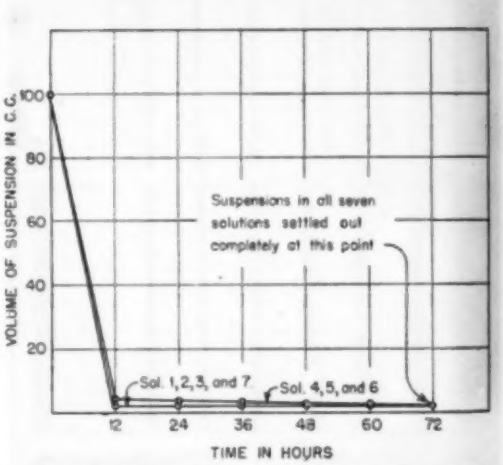

Fig. 8.—Stability of fuller's earth suspensions 2% in media of varied *p*H.

Figures 2 and 3 show that all of the suspensions of bentonite are less stable in acidic solutions and at high *p*H values and that the optimum *p*H range at which they are stable is 6.9–8.9.

Figure 4 shows that the settling of the so-called activated bentonite, prepared according to the procedure of Maynard (12), is rapid and almost complete in all suspensions. This may be explained by the fact that in the preparation of this form it was washed with diluted mineral acid, which apparently neutralizes the surface hydroxyl ions of the bentonite, thereby destroying its suspending properties

Figures 4 and 5 indicate that kaolin suspensions are unstable in almost any *p*H. Fuller's earth acts in the same manner (Figs. 7 and 8).

Figures 9 and 10 show the comparative stability of 2% suspensions of the clays in distilled water during one hundred and forty-four hours.

This investigation indicates that, since bentonite is official as a suspending agent, the pharmacopœial monograph should establish certain requirements pertaining to its suspending property to distinguish it from other clays. The following recommendation is proposed:

Stability of the Suspension:

"A suspension of bentonite (2 Gm. per 100 cc.) in distilled water contained in a tube, 2 cm. in diameter and 30 cm. tall, shall remain in suspension for not less than forty-eight hours with not more than a layer of 0.5 cm. of bentonite settling out in this period of time."

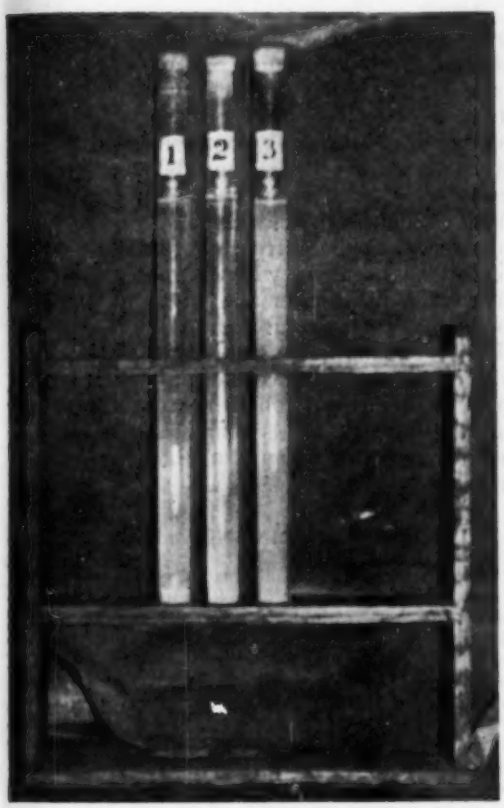

Fig. 9.—Comparative Clay Suspensions in Distilled Water after standing 144 hours. No. 1 is Fuller's Earth; No. 2, Kaolin; and No. 3, Bentonite.

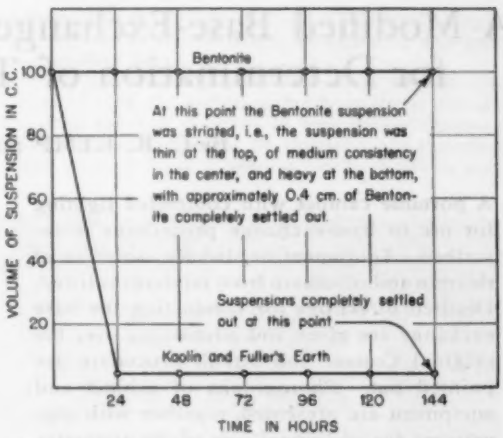

Fig. 10.—Comparative stability of 2% suspensions of kaolin, fuller's earth and bentonite in distilled water.

CONCLUSIONS

1. The unusual suspending properties of bentonite are due, chiefly, to its singular lattice-like crystalline structure and only to a minor degree to its small particle size.
2. Since kaolin and fuller's earth do not exhibit this lattice-like structure, they are distinctly inferior to bentonite as a suspending agent.
3. The *p*H of the outer phase has a marked effect on the stability of clay suspensions which, particularly in the case of bentonite, are more stable in neutral and alkaline mediums than in acid mediums.
4. The identification test in the Pharmacopœia is only an approximate one.
5. A procedure for estimating the stability of suspensions of bentonite is outlined and recommended for inclusion as a means of the identification of bentonite.

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A Modified Base-Exchange Procedure and Apparatus for Determination of Thiamin and Riboflavin*

By C. R. KEMP and F. J. BANDELIN

A portable cabinet with controlled lighting for use in base-exchange procedures is described. Equipment needed for isolation of thiamin and riboflavin from mixtures is listed. Detailed directions for conducting the base exchange are given and advantages over the original Conner and Straub procedure are pointed out. Photographs of cabinet and equipment are presented, together with suggestions for additional uses of the apparatus in routine laboratory procedures.

THE acceptance of thiochrome method as official for assay of thiamin in pharmaceutical products (1) will undoubtedly stimulate the use of base-exchange procedures. Many laboratories not equipped for bioassay may now make use of the thiochrome method for control work.

Details of equipment necessary for the thiochrome method are not given in the U. S. P. Neither does it give specific directions for carrying out the determination. Such information as is available is widely scattered throughout the literature. The original method of Jansen (2) has been repeatedly modified, and there is a need for a current review of apparatus and techniques involved in the thiochrome method of thiamin assay. This paper will be limited to a consideration of the base-exchange procedure. Subsequent papers will consider preparation of pharmaceutical samples for assay, the factors involved in the oxidation of the thiamin to thiochrome, and the use of the fluorophotometer for measuring the resulting fluorescence.

The method of Jansen was modified by Kubli (3) and later by Hennessy and Cerecedo (4), who first utilized the synthetic zeolite, Decalso, as the medium of isolating thiamin from impure samples. The latter investigators also introduced the fluorophotometer (Pfaltz and Bauer) for measurement of the fluorescence of the thiochrome.

Many workers have modified the method of Hennessy and Cerecedo both in terms of apparatus and in terms of specific techniques, but the essential features of their method have been changed but little. A widely used modification is that of Conner and Straub (5) who introduced specialized equipment designed to facilitate the digestion of samples and the subsequent base exchange. In an accompanying paper (6) Conner and Straub further modified the apparatus and procedures for thiamin determination and introduced a combined procedure for isolating both thiamin and riboflavin concurrently from a mixture.

Much of the work as presented by Conner and Straub still stands, but the apparatus and techniques presented by them offer certain opportunities for modification. This is especially true for those laboratories where thiamin and riboflavin determinations are done on small numbers of samples and on other than a continuous or daily basis. Such is the case in the average-sized pharmaceutical control laboratory or in a research laboratory where samples are relatively few in number and where determinations may be separated by some period of time. The apparatus described below obviates the need for a permanent installation requiring controlled lighting in the entire laboratory; it also eliminates the problem of periodic assembling of an unstable ironware setup.

EXPERIMENTAL

The unit used in our laboratory is constructed of 1/4-inch plywood (Figs. 1, 2, 3) and is in the form of an enclosed cabinet with an inclined front consisting of hinged doors. The windows are covered with amber-colored Monsanto Fibestos[1] (cellulose acetate sheet) 0.03 inch in thickness. The doors are so constructed that either half of the upper portion may be opened for regulation of the rate of flow of solutions through the exchange columns; the door, as a whole, can be folded and lowered to form a convenient shelf or platform in front of the cabinet when exchanging apparatus or at any time when light exclusion is not a factor (Figs. 2, 3).

On the base of the cabinet are mounted two standard bulb sockets wired in parallel, the cord extending through the back of the cabinet. Two 8 c. p., 115 v., amber safe light bulbs are used for illumination whenever necessary. The unit, therefore, makes controlled lighting of the entire laboratory unnecessary if riboflavin determinations are being done; in addition, the light sources give adequate local lighting when the unit is used at

* Received March 2, 1945, from the Research Laboratories, Flint, Eaton & Company, Decatur, Ill.

[1] Monsanto Chemical Co., Plastics Division, Springfield, Mass.

night or in poorly lighted positions in the laboratory.

The inside dimensions of the cabinet are as follows: top, 40 x 5 inches; base, 40 x 10 inches; height, 19 inches. Beginning at a level one inch above the base, the front slopes to meet the top of the cabinet. The resulting inclination, approximately 15 degrees from the vertical, allows convenient visibility of the interior. Handles mounted on either end of the unit make it readily portable; thus, the unit can be used anywhere in the laboratory or it can be set aside when not in use.

On the inside of the back of the unit, two inches below the top, is mounted a vacuum manifold[2] with four stopcocks. A length of medium-heavy pressure tubing extends through a hole in the upper left end of the cabinet and is attached to any convenient filter pump.

On a line 9 inches above the base and at distances of 5, 15, 25, and 35 inches from either end are mounted the jaw portions of four utility clamps.[3] The clamps are mounted by thrusting the threaded stems through $^3/_8$-inch holes in the back of the unit and by affixing the lock nuts on the outside of the back. That jaw of the clamp opposite the adjusting nut is ground down to fit it between the side arm and the rim of a 250-ml. suction flask. The clamps are mounted with the adjusting nut to the right as viewed from the front of the cabinet.

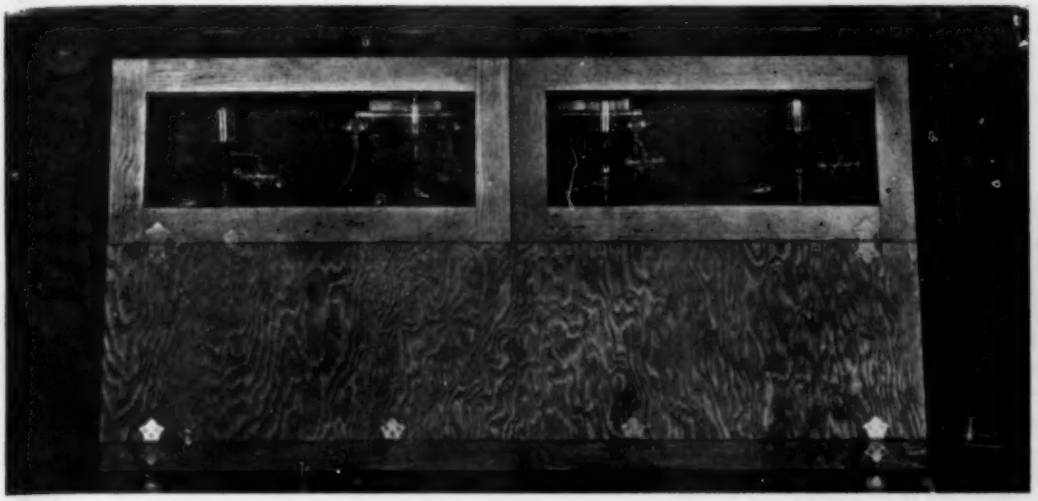

Figure 1

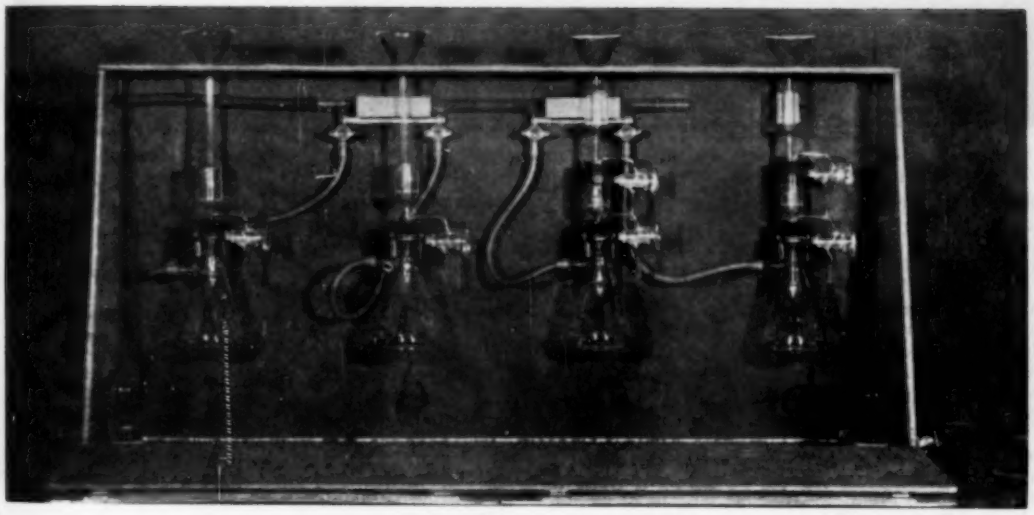

Figure 2

[1] Scientific Glass Apparatus Co., Bloomfield, N. J., Catalog No. J-6040.

[2] Fisher Scientific Co., Pittsburgh, Pa., Catalog No. 5-766.

Through the top of the cabinet, midway between the front and back, are four 1-inch holes. The positions of the holes with reference to the ends of the unit coincide with those of the clamps below. The diameter and position of the holes are such that 60-degree funnels rest securely, and the stems extend along the sides of the base-exchange column reservoirs below.

The procedure of Conner and Straub has been modified for convenience and economy of effort. By using 250-ml. suction flasks in the absorption stage, the need for removing, washing, and drying the vacuum receivers during the determination is eliminated. All elution is done directly into the graduated vacuum receivers instead of into small suction flasks, thus allowing accurate measurement of the volume of eluate. Since the volume of the eluate determines the concentration of the substance under determination, this point is important. Another point of convenience is that only one size of stopper is needed throughout the determination; a No. $6^1/_2$ rubber stopper will fit the top of the 250-ml. suction flask, the top of the vacuum receiver, and, if riboflavin is to be adsorbed concurrently, the top of a second base-exchange tube. Since the degree of negative pressure needed to maintain an even flow through the columns is low, the fit of the stoppers need not be perfect.

The adsorption phase of the base exchange is carried out as follows: 250-ml. suction flasks are placed in each clamp (Fig. 2, *a*, *b*, *c*, *d*) and are connected to the vacuum manifold by short lengths of flexible tubing. Pressure tubing need not be used. For adsorption of thiamin or riboflavin, alone, a No. $6^1/_2$ stopper is fitted with an exchange column[4] and a throttling stopcock[5] and is inserted into the neck of the suction flask (Fig. 2, *a*, *b*). "Policemen" may be placed over the tips of the exchange columns to keep them filled with water until ready for use. The rubber bulbs from ordinary dropper pipettes have proved to be well suited for this purpose. A long-stemmed funnel will just reach the midpoint of the reservoir when the tip of the column is properly located with reference to the side arm of the flask. If both thiamin and riboflavin are present and are in proper proportion, they may be adsorbed concurrently by placing a second exchange unit in the top of the first one (Fig. 2, *c*, *d*) as outlined by Conner and Straub (6). A No. $6^1/_2$ stopper will also serve here, and, after absorption, the exchange unit may be transferred directly to the top of a vacuum receiver for elution. A short-stemmed funnel will, in this case, reach a median point in the upper exchange column reservoir.

A problem of inequality of rate of flow through the two columns may arise if dual adsorption is attempted. The problem of keeping both columns of adsorbent covered with fluid is readily controlled if the lower column drains less readily than the upper. In this case the upper throttling stopcock is opened, thus decreasing the rate of flow of the upper column. However, if the lower column drains more readily than the upper, a problem arises. Since it is apparently essential to keep the fluid level above the surface of the adsorbent, the authors have resorted more often to separate absorption columns rather than to the dual assembly. The slight gain in time resulting from dual adsorption seems more than offset by the added problem of controlling the rate of flow and of transferring the adsorption columns for subsequent elution.

When the last portion of the assay sample has almost reached the upper surface of the adsorbent, the first of three 5-ml. portions of hot, distilled water is added. Each subsequent wash portion is added before the reservoir runs completely dry. When about one-half of the last wash portion of water is still above the adsorbent, the suction flask with its entire assembly is removed from the clamp. A vacuum receiver, graduated to 50 ml.,[6] is placed in

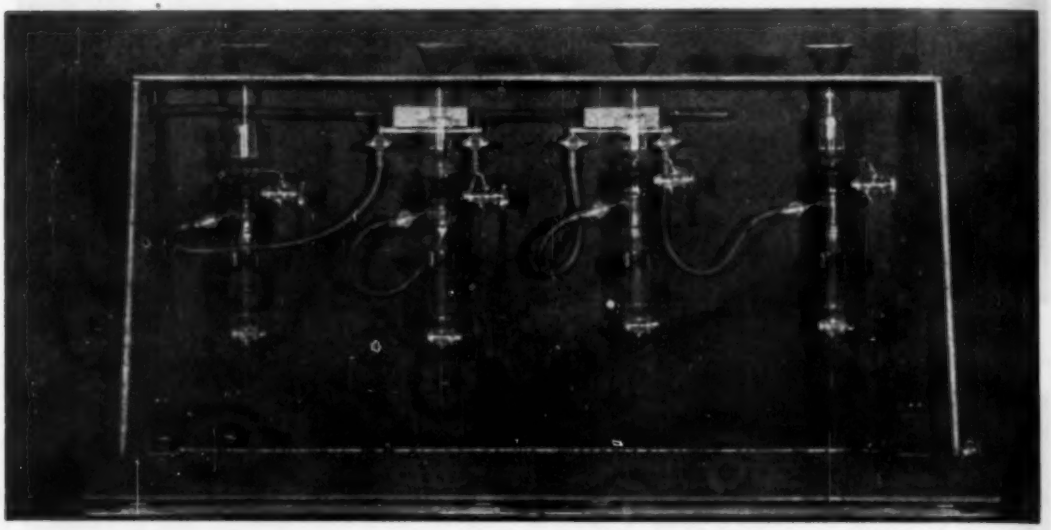

Figure 3

[4] Scientific Glass Apparatus Co., Bloomfield, N. J., Catalog No. J-6035.

[5] Scientific Glass Apparatus Co., Bloomfield, N. J., Catalog No. J-6041.

[6] Scientific Glass Apparatus Co., Bloomfield, N. J., Catalog No. J-6050.

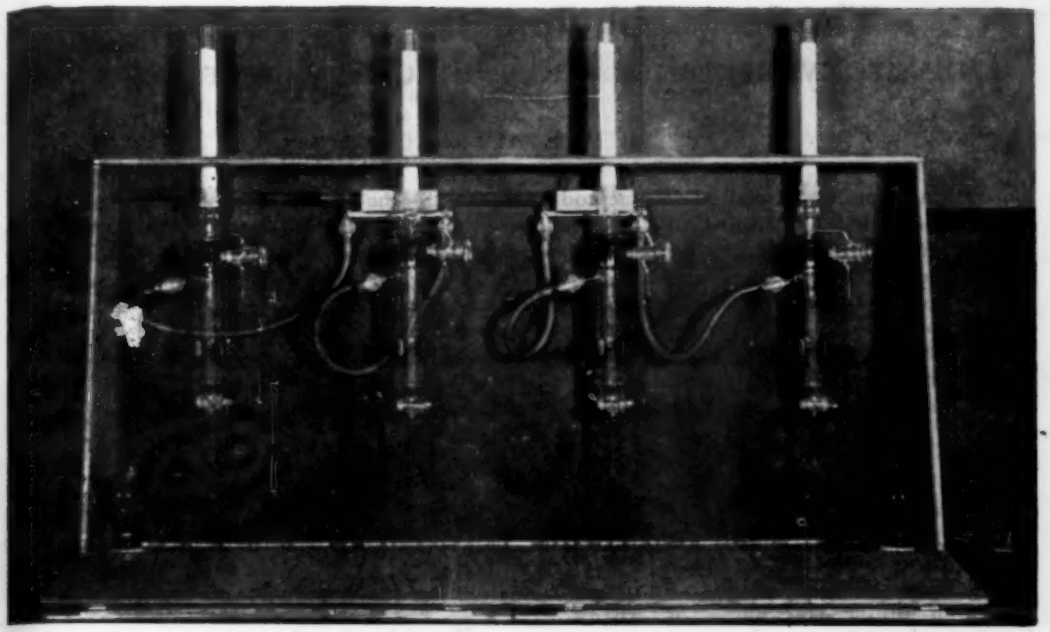

Figure 4

the clamp and connected to the tubing leading to the vacuum manifold (Fig. 3). The exchange column unit is now transferred to the top of the vacuum receiver and a short-stemmed funnel substituted for the longer one used in the adsorption phase. The eluent is added in small portions until the required volume is collected in the vacuum receiver. Care must be exercised to keep the eluent above the surface of the adsorbent; however, each portion must be nearly expelled before the succeeding portion is added if thorough washing of the assay material from the adsorbent is to be achieved.

Since the diameter of the vacuum receiver is quite great, removal of the small amount required for oxidation to thiochrome (5 ml. or less) through the stopcock at the bottom is impractical due to the error involved. It is best transferred by pipette to the oxidation reaction vessel.[7] Care must be taken to mix the eluted fluid thoroughly before the transfer, since the first portions of the eluent received in the vacuum receiver are richer in assay material than the latter portions. Because of this need for pipetting the aliquot of the eluent into the reaction chamber for oxidation, an ordinary side arm test tube graduated at any desired level may be substituted for the more expensive vacuum receiver. The advantage of having a receiver capable of taking a No. $6\frac{1}{2}$ stopper and graduated progressively from zero to 50 ml. is obvious, however. Where samples of widely different vitamin content are encountered, the adjustment of final volume of eluent may conveniently serve as one of the required dilution factors required to bring the final sample within the working range of the fluorophotometer.

The authors have also found the apparatus described above useful in the evaporation of solvents from solutions by passing the stream of gas into the manifold and directing it into each reaction unit by means of the separate rubber tubes. In general, many reactions requiring an inert atmosphere may be carried out in a similar manner by connecting the supply tank of inert gas to the manifold. Another use has been made of the cabinet by utilizing it as a support for chromatographic columns (7, 8) (Fig. 4). A series of four columns may be readily controlled by thrusting the columns through the holes prepared for the funnels and then inserting them in the vacuum receivers equipped with throttling stopcocks. The control of the degree of negative pressure is an asset. The graduations of the vacuum receiver, together with the stopcock in the bottom which permits successive portions of the eluent to be withdrawn without dissembling the setup, makes the production of liquid chromatograms an easy task.

An added asset of the cabinet is that specialized equipment required for vitamin assay or any other particular determination may be kept in it between periods of use. Such materials will be readily available when required and will be kept clean and free from contamination.

[7] Scientific Glass Apparatus Co., Bloomfield, N. J., Catalog No. J-6025 or J-6026.

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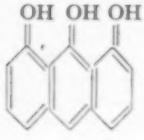
The Spectrophotometric Estimation of Dihydroxyanthranol and Dihydroxyanthraquinone*

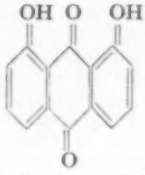
By M. E. AUERBACH

A simple spectrophotometric method is presented for the analysis of 1,8-dihydroxy-9-anthranol (Anthralin) and 1,8-dihydroxy-9,10-anthraquinone (Danthron), either individually or in admixture. It is particularly advantageous for the assay of commercial dihydroxyanthranol inasmuch as it permits the simultaneous determination of 1,8-dihydroxyanthraquinone, a frequent and natural contaminant. When single analyses are made, the average error of the method amounts to about ±2 per cent. Exceptionally, the error involved for the minor component may amount to 10 per cent or more. If special interest is attached to the content of the minor component, the analysis should be run in triplicate and calculations made from the average observed extinction value.

1,8-Dihydroxy-9-anthranol and its primary oxidation product, 1,8-dihydroxy-9,10-anthraquinone, are each pharmaceuticals of established value. The former, available under the name of Anthralin, has been used for some years in the treatment of psoriasis (1) and related dermatoses. The latter, known as Danthron, is an effective purgative (2), used largely in veterinary medicine.



1,8-dihydroxyanthranol



1,8-dihydroxyanthraquinone

The anthranols are potent reducing agents, and, indeed, the dermatological value of dihydroxyanthranol is associated with this reducing action. The quantitative analysis of this substance properly concerns itself with determining how much of the sample is in the anthranol form and how much in the inactive anthraquinone form. The assay method recently described (3) by the Council on Pharmacy and Chemistry depends upon the reduction of ammoniacal silver nitrate and the subsequent gravimetric estimation of metallic silver. The particular advantage of this method is that it requires no special apparatus. However, it leaves much to be desired in elegance and selectivity. The present paper describes a spectrophotometric method which is reliable, requires little manipulation, and permits the simultaneous estimation of any 1,8-dihydroxyanthraquinone which may be present.

* Received March 19, 1945, from the Research Laboratories of the Winthrop Chemical Company, Inc., Rensselaer, N. Y.

EXPERIMENTAL

Materials.—1. Commercial chrysazin (1,8-dihydroxyanthraquinone) was recrystallized repeatedly from ethylene dichloride. The final crop of reddish yellow crystals melted at 191.5°–192.5° C. corr. Naylor and Gardner (4), who prepared this substance with special care, give the melting point as 192°–193° C. when crystallized from 50% alcohol.

2. A commercial sample of Anthralin prepared by the reduction of chrysazin, was crystallized twice from ethylene dichloride. The final product consisted of yellow plates melting at 178°–179° C. corr. Further crystallization from glacial acetic acid did not change the melting point. Schrobsdorff (5) gives the melting point of this substance as 176°–177° C. when crystallized from ligroin.

Method.—About 200 mg. of sample, consisting of dihydroxyanthranol, dihydroxyanthraquinone, or a mixture of the two, are dissolved in C. P. benzene and brought to a concentration of about 10 mg. per liter by appropriate successive dilutions. The extinction value of the final dilution is then determined in a spectrophotometer at each of two wave lengths, 358 mμ and 432 mμ. These wave lengths, as shown in Fig. 1, correspond to the absorption maxima of dihydroxyanthranol and dihydroxyanthraquinone, respectively. All of the absorption data recorded in this paper were obtained by the use of the Beckman quartz spectrophotometer (6), employing 1-cm. cells.

TABLE I.—$E^{1\%}_{1\ cm.}$ VALUES AT SIGNIFICANT WAVE LENGTHS

λ	1,8-Dihydroxy-9-anthranol	1,8-Dihydroxy-9,10-anthraquinone
310	197	107
340	358	54
358	455	83
432	13	448
455 (inflection)	...	331

Calculation of Results.—Absorption data of analytical significance are recorded in Table I. It should be noticed that the respective points of maximum absorption are well separated, with only minor interference in each case. In mixtures, such as represented by this pair, the calculation of the concentration of each component is readily accomplished. The formulas given below are adapted from the recent monograph by J. R. Loofbourow (7).

$$C_1 = \frac{k_2D' - k_2'D}{k_1'k_2 - k_1k_2'}$$

in which

C_1 = concentration of 1,8-dihydroxyanthranol
D = total density reading at 358 mμ
D' = total density reading at 432 mμ
$k_1 = E^{1\%}_{1\text{ cm.}}$ (358 mμ) for 1,8-dihydroxyanthranol
$k_2 = E^{1\%}_{1\text{ cm.}}$ (358 mμ) for 1,8-dihydroxyanthraquinone
$k_1' = E^{1\%}_{1\text{ cm.}}$ (432 mμ) for 1,8-dihydroxyanthranol
$k_2' = E^{1\%}_{1\text{ cm.}}$ (432 mμ) for 1,8-dihydroxyanthraquinone

Then

$$C_1 = \frac{83D' - 448D}{(13)(83) - (455)(448)} = \frac{5.4D - D'}{2440}$$

Similarly,

$$C_2 = \frac{k_1D' - k_1'D}{k_1k_2' - k_1'k_2}$$

in which C_2 = concentration of 1,8-dihydroxyanthraquinone and all other symbols as given.

Then

$$C_2 = \frac{455D' - 13D}{(455)(448) - (13)(83)} = \frac{35D' - D}{15{,}600}$$

TABLE II.—EXTINCTION VALUES OF SOLUTIONS OF VARYING CONCENTRATION

Mg./L. Benzene	1,8-Dihydroxy-9-anthranol $E_{1\text{ cm.}}$ (358 mμ)	1,8-Dihydroxy-9,10-anthraquinone $E_{1\text{ cm.}}$ (432 mμ)
4.0	0.180	0.186
8.0	0.358	0.363
12.0	0.540	0.535
16.0	0.718	0.718
20.0	0.898	0.888

Table II presents actual data obtained in this laboratory for several concentrations of dihydroxyanthranol and dihydroxyanthraquinone, respectively. In each case, if extinction value is plotted against concentration a straight line results. That is to say, solutions of the individual pure compounds conform to Beer's law in the indicated range of concentration.

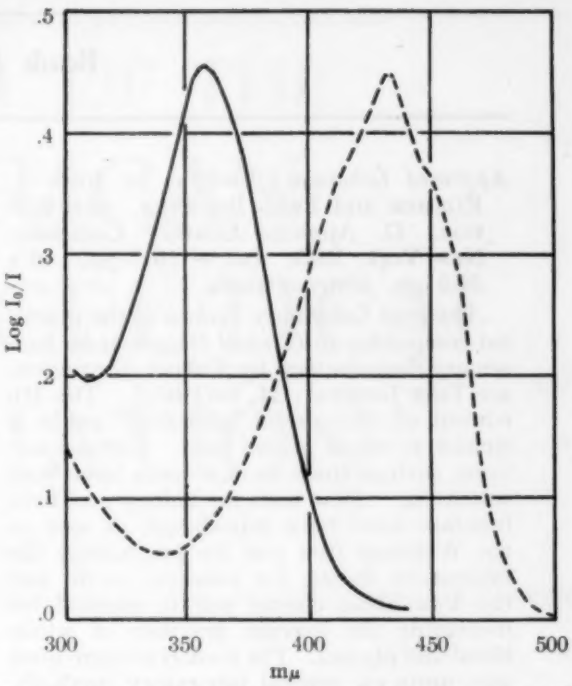


Fig. 1.—Spectral Absorption of Anthralin ————; of Danthron - - - - -; Solvent—; Benzene; Concentration—0.001%

Table III sets forth the observed E values of five trial mixtures of dihydroxyanthranol and dihydroxyanthraquinone. The calculated results were derived by application of the recommended formulas.

TABLE III.—THE RESOLUTION OF MIXTURES OF 1,8-DIHYDROXYANTHRANOL (I) AND 1,8-DIHYDROXYANTHRAQUINONE (II) BY SPECTROPHOTOMETRIC ANALYSIS

Trial Mixtures,[a] Mg.		Observed $E_{1\text{ cm.}}$ Values		Found, Mg.	
I	II	358 mμ	432 mμ	I	II
1.000	0.050	0.458	0.042	0.996	0.065
1.000	0.250	0.464	0.128	0.974	0.257
0.500	0.500	0.271	0.231	0.505	0.500
0.250	1.000	0.199	0.453	0.254	1.003
0.050	1.000	0.106	0.445	0.052	0.991

[a] Each trial mixture is dissolved in 100 cc. benzene.

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Book Reviews

Approved Laboratory Technic, by JOHN A. KOLMER and FRED BOERNER. 4th Edition. D. Appleton–Century Company, New York, 1945. lxii + 1017 pp. 16 x 24.5 cm. Price, $10.00.

Approved Laboratory Technic is the practical companion to *Clinical Diagnosis by Laboratory Examinations* by Kolmer [for review, see THIS JOURNAL, **34,** 64(1945)]. The 4th edition of this useful laboratory guide is similar to those of the past. Certain sections, such as those on skin tests, have been rewritten. New tests for kidney and liver function have been introduced, as well as the Weisman frog test for pregnancy, the fluorescent dyeing for tubercle bacilli and the Van Slyke copper sulfate method for measuring the specific gravities of whole blood and plasma. The book is broken down into units on general laboratory methods, clinical pathological methods, bacteriological, mycological and virological methods, parasitological methods, serological methods, chemical methods, and pathological methods.

No more complete and authoritative book of clinical laboratory methods is available in the United States. It should be on the book shelf of every hospital pharmacist.—MELVIN W. GREEN

A Manual of the Aspergilli, by CHARLES THOM and KENNETH B. RAPER. The Williams & Wilkins Company, Baltimore, 1945. ix + 373 pp. 76 illus. Price $7.00.

Although the aspergilli have been an interesting and useful group of microbes for many years, the discovery of the antibiotics has focused the attention on them that they deserve.

The value of this manual lies in the fact that the authors have made consistent efforts "to obtain the organisms actually used by the authors who have put forward new nomenclature." Where information available does not justify the allocation to some species, the name will be found in a check list of such "species" in the back portion of the book. The authors have taken great pains to create a manual which serves to identify molds simply, consistently, and accurately. An index of its scholarliness and authoritativeness lies in the list of international collaborators in the preface.

The first part of the manual is given over to a general discussion of classification, morphology, cultivation, examination, preservation of culture, and variation, and includes in addition an interesting historical introduction. That the manual is up to date in this category is indicated by the inclusion of methods of lyophilozation.

The second portion of the book constitutes "The Manual Proper." After a chapter on the use of the manual, the individual groups of aspergilli are discussed. The many fine illustrations, especially those in color, are helpful to the laboratory worker in reaching a decision on identification. In addition, the occurrence, economic importance, and antibiotic activity are outlined under each phylogenic grouping.

The reference material, constituting Part III, is also very valuable. Herein is included a "Topical Bibliography" of 30 pages containing references broken down into 33 subheadings. Ten additional pages of "General Bibliography" make the documentation complete and useful.

"A Check List of Species and Genera" (29 pages) and a list of "Accepted Species, Varieties and Mutations" (2 pages) complete the manual.

The typography, illustrations, and qualities of materials make this manual a real tribute to the printer's art.—MELVIN W. GREEN.

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